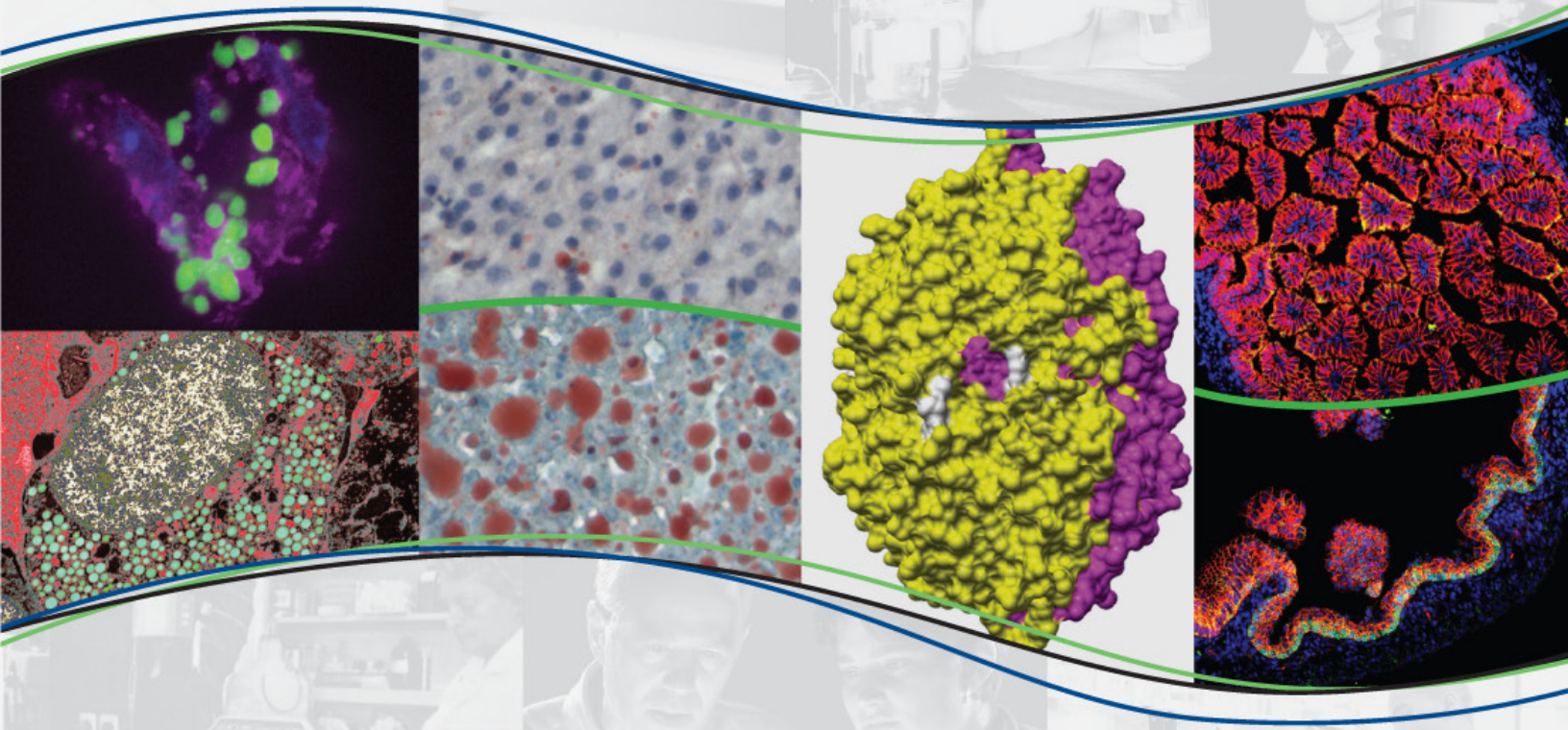
NIDDK

Recent Advances and Emerging Opportunities

February 2010



60 years 1950 2010 NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes & Digestive & Kidney Diseases

Cover color images: The color images represent some of the compelling NIDDK-supported research advances of the past year in different topic areas within the Institute's mission. More information on these and other exciting research findings can be found throughout this compendium.

Cover NIDDK logo and background images: The Institute celebrates its 60th Anniversary in 2010 and gratefully acknowledges and applauds the efforts and creativity of the highly talented and dedicated scientists whose critical discoveries over the last 6 decades have improved the Nation's health.

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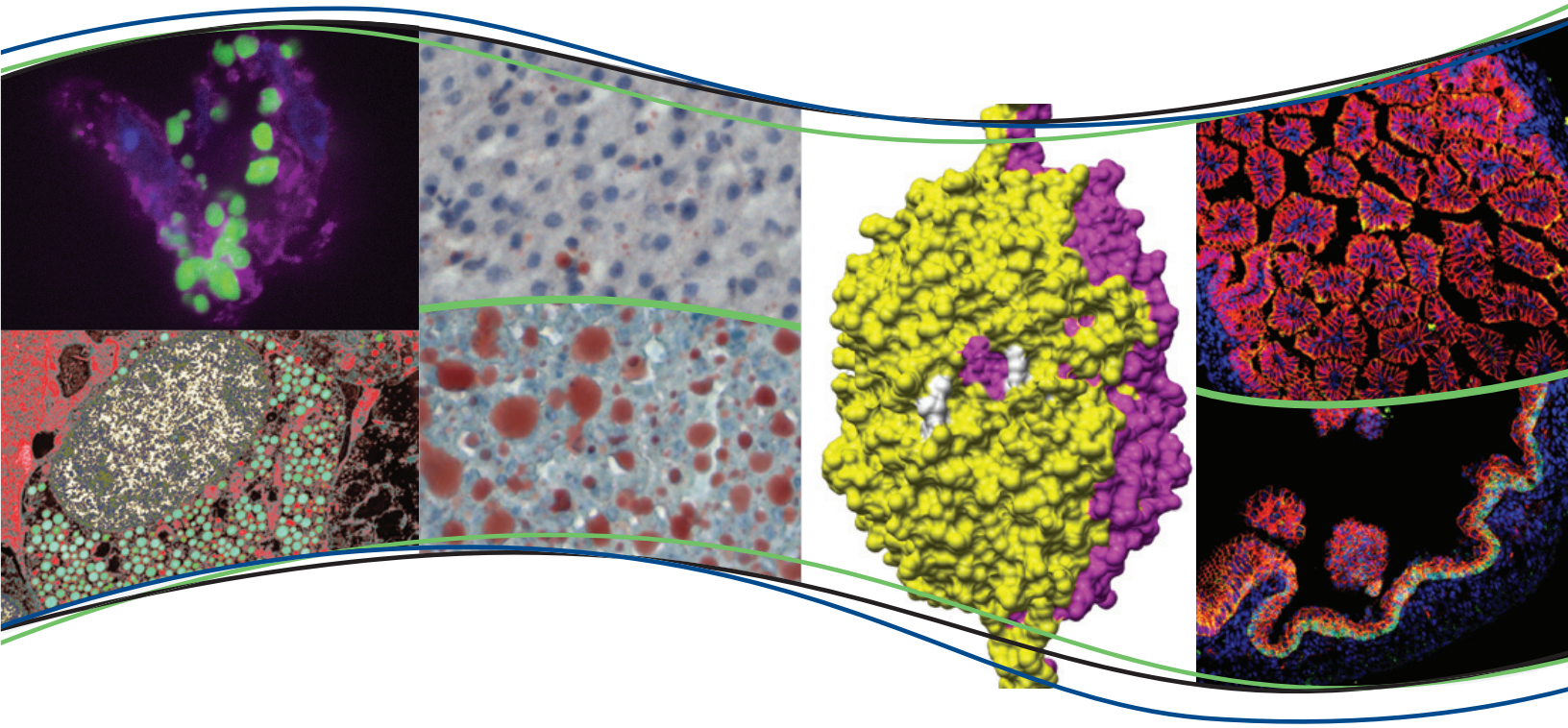

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CONTENTS

Message from the Director

As the Director of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), I am pleased to present this annual compendium highlighting the research efforts and programs supported by the Institute. The NIDDK has a broad research responsibility, which includes some of the most common, debilitating, and costly conditions affecting Americans. These conditions include diabetes and other endocrine and metabolic diseases, such as cystic fibrosis; liver disease and other digestive diseases, such as inflammatory bowel disease; nutritional disorders and obesity; kidney diseases, such as polycystic kidney disease; urologic diseases, such as interstitial cystitis/painful bladder syndrome; and hematologic diseases, such as Cooley's anemia.

This 10th edition illustrates recent NIDDK-supported scientific advances, such as:

- Identification of more than 40 genes or regions of the genome that affect risk of type 1 diabetes;
- Revelation that an energy-burning form of fat tissue is active in adults;
- Discovery of the protein target of the diabetes drug metformin;
- Finding that the risk of short-term complications is low after bariatric surgery for extreme obesity;
- Insights into how certain types of bacteria found in the human gut—and their collective genomes—may contribute to their host's obesity or leanness;
- Identification of new genetic risk factors associated with ulcerative colitis;
- Demonstration that weight loss in overweight or obese women reduces urinary incontinence;
- Finding that people who donate a kidney have similar long-term survival and quality of life as the general population; and
- Identification of key factors that interact to regulate blood stem cell development.

This compendium also includes personal stories of patients. A family dedicated to advancing research on type 1 diabetes describes its participation in a clinical trial testing an agent to delay or prevent the disease. A man who participated in the landmark Diabetes Prevention Program that showed type 2 diabetes can be prevented or delayed with modest weight loss continues to be followed to determine the long-term effects of the intervention. A woman shares her story and warns about acute liver failure caused by over-the-counter medication. A woman who participated in a study testing the effects of weight loss on urinary incontinence reveals how the study positively affected her life.

The NIDDK is continuing efforts to ensure that knowledge gained from its research advances is disseminated to health care providers, patients, and the general public. Such efforts include the Institute's major educational programs, the National Diabetes Education Program and the National Kidney Disease Education Program. Additionally, the Weight-control Information Network, the National Diabetes Information Clearinghouse, the National Digestive

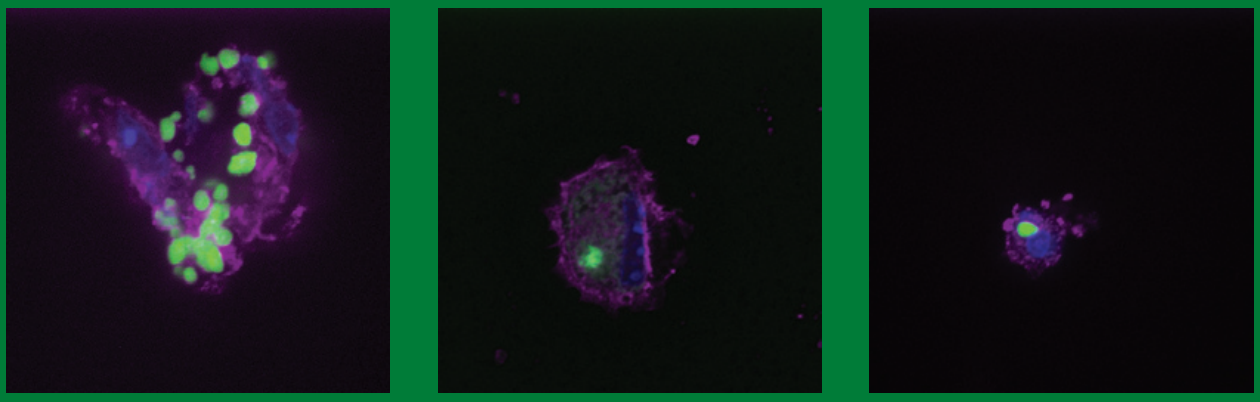
Diseases Information Clearinghouse, and the National Kidney and Urologic Diseases Information Clearinghouse develop and distribute science-based information on diseases and disorders within the NIDDK mission. Several hundred brochures, fact sheets, and publications are available in printed copy and on the NIDDK Web site so that they are readily available for patients, health care providers, and the public. I invite you to visit the Web site at: *www.niddk.nih.gov*

This compendium reflects only a fraction of the immense body of research performed by basic scientists, clinical investigators, and patient volunteers. We remain committed to translating their efforts into improvements in the health and quality of life of all people.

Griffin P. Rodgers, M.D., M.A.C.P.
Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
U.S. Department of Health and Human Services

The materials featured in this publication reflect the core mission of the NIDDK, including the Director's following guiding principles:

- Maintain a vigorous investigator-initiated research portfolio;
- Support pivotal clinical studies and trials;
- Preserve a stable pool of talented new investigators;
- Foster exceptional research training and mentoring opportunities; and
- Ensure knowledge dissemination through outreach and communications.



Research has revealed a link between obesity and the inflammatory response of the immune system. As the body takes in excess calories, fat cells increase in size to store the extra fat. Eventually the fat cells become overloaded and begin to release molecules that attract inflammatory cells, specifically macrophages. When macrophages are continually recruited to the fat cells, as is the case in obesity, a chronic state of inflammation can occur, contributing to insulin resistance and type 2 diabetes.

NIDDK-supported scientists recently sought to apply "RNA interference" to modulate the functioning of macrophages, so as to dampen inflammation. RNA interference, discovered previously by other researchers, involves reducing the levels of specific proteins with molecules called small interfering RNAs or siRNAs. This revolutionary technique has enormous therapeutic potential, but its application has been limited by lack of an effective and safe oral delivery vehicle. In a novel approach toward overcoming this obstacle, NIDDK-supported scientists developed a delivery vehicle called glucan-encapsulated siRNA particles, or GeRPs. When GeRPs (shown in green) were fed to mice, they were taken up by macrophages (shown in magenta) and migrated to several different tissues. These images illustrate the presence of GeRPs in mouse spleen (left), liver (middle), and lung (right) tissues. By feeding mice GeRPs with siRNA to a specific inflammatory protein, the scientists were able to reduce levels of this protein and to suppress inflammation in mice. Because macrophages and inflammation are involved in many diseases, developing a strategy for therapy in the macrophages might be applied to many other diseases. As described in this chapter and elsewhere in this book, NIDDK-supported research is making advances in understanding and treating numerous NIDDK-related diseases.

Image provided by Dr. Michael P. Czech and reprinted by permission from Macmillan Publishers Ltd: Nature 458: 1180-1184, copyright 2009.

Cross-Cutting Science

Advances in medicine are largely dependent upon the accumulation of new knowledge about biologic processes, often at the smallest levels of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other. Major strides in fighting disease can be traced back to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. Opportunities to make exciting new discoveries are arising ever more rapidly with the development of new technologies, new approaches, and even new scientific disciplines as teams of talented, creative researchers join together to pursue increasingly complex challenges. Described in this chapter are several recent studies, each of which spans multiple areas within the NIDDK research mission. The insights gained through this kind of research can be expected to aid progress in many scientific endeavors, for today's research advances may lead to tomorrow's cures.

NIDDK CELEBRATES ITS 60TH ANNIVERSARY

In 2010, the NIDDK celebrates 60 years since its founding. Over the course of its history, the Institute that is known today as the National Institute of Diabetes and Digestive and Kidney Diseases is proud to have supported and conducted research on many of the most serious diseases affecting public health. Affecting people of all ages and ethnic groups, the diseases within the NIDDK research mission encompass some of the most common, severe, and disabling conditions, as well as less prevalent but nonetheless debilitating diseases, affecting Americans today: endocrine and metabolic diseases and disorders such as diabetes and obesity, digestive diseases such as hepatitis and inflammatory bowel disease, kidney and urologic diseases such as kidney failure and prostate enlargement, and blood diseases such as the anemias.

The research advances made possible through 60 years of NIDDK support have saved lives, improved quality of life, and laid the foundation for future progress. The Institute has supported a number of winners of the world's greatest scientific honor. Many have won the Nobel Prize in Physiology or Medicine, and others have received the Nobel Prize in Chemistry. These include extramural scientists at universities and other research institutions across the country who have been supported by the NIDDK (Institute grantees), as well as scientists within the Institute's Division of Intramural Research.

As part of a year-long celebration to mark its 60th anniversary, the Institute has planned a variety of activities. Scientific symposia are planned for the annual meetings of several professional societies and organizations with a focus on diseases within the Institute's mission. The NIDDK is also publishing a booklet for the public that commemorates its 6 decades of support for biomedical research and highlights discoveries that have been made by NIDDK-funded investigators and NIDDK intramural scientists. The anniversary celebrations will additionally feature an NIDDK Anniversary Scientific Symposium on the National Institutes of Health (NIH) campus in September 2010.

History of the NIDDK: On August 15, 1950, President Harry S. Truman signed into law the Omnibus Medical Research Act, establishing the National Institute of Arthritis and Metabolic Diseases (NIAMD)—which would become today's NIDDK. The new Institute incorporated the laboratories of the Experimental Biology and Medicine Institute and expanded to include clinical investigation in rheumatic diseases, diabetes, and a number of metabolic, endocrine, and gastrointestinal diseases. That same year, the NIAMD Advisory Council held its first meeting and recommended approval of NIAMD's first grants. In November 1950, U.S. Surgeon General Leonard Scheele formally established NIAMD.

Over the years, the NIAMD evolved into the National Institute of Arthritis, Metabolism, and Digestive Diseases (in 1972) and the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (in 1981). In 1986, the Institute's Division of Arthritis, Musculoskeletal and Skin Diseases became the core of a new, independent Institute. The NIDDK then acquired its current name, the National Institute of Diabetes and Digestive and Kidney Diseases.

AMERICAN RECOVERY AND REINVESTMENT ACT OF 2009 AND NIH BIOMEDICAL RESEARCH

The American Recovery and Reinvestment Act of 2009 (also referred to as ARRA or the Recovery Act) was signed into law by President Barack Obama on February 17, 2009. The legislation provides a total of $10.4 billion to the NIH: $8.2 billion in extramural funding for scientific research; $1 billion to the National Center for Research Resources (NCRR) to support extramural construction, repairs, and alterations in support of all research institutions that receive NIH funding; $300 million for shared instrumentation and other capital equipment to support all NIH activities; $500 million for high priority repair, construction, and improvement projects on NIH campuses; and $400 million to support comparative effectiveness research. The NIDDK received $445 million of these funds, as well as additional funds from the Office of the NIH Director and NIH Common Fund allocations.

The NIDDK is deploying funds made available through the Recovery Act to support highly meritorious applications for research projects, to target supplements to accelerate the pace of ongoing science, and to fund new NIH activities, such as Challenge Grants. The Institute is working closely with the broader NIH community and the U.S. Department of Health and Human Services to ensure that scientific merit, as well as process transparency and accountability, are the guiding principles behind the Institute's implementation of the Recovery Act.

- For an overview of the Recovery Act, visit: *www.recovery.gov/*
- For more information about the Recovery Act at NIH, visit: *www.nih.gov/recovery*
- For more information on NIDDK's implementation of the Recovery Act, see *www2.niddk.nih.gov/Recovery/*

RECENT GENETICS STUDIES: PAVING A WAY TOWARD IMPROVING PEOPLE'S HEALTH

New research resources, including the wealth of information from the Human Genome Project and the International HapMap project, are making it easier for scientists to identify genes that influence a person's likelihood of developing a variety of genetically complex diseases. Ranging from rare conditions to very common diseases such as type 2 diabetes, these diseases result from variations in multiple genes. Because these variations may individually have only modest contributions to disease susceptibility—along with other genetic and environmental factors—it has been challenging to identify the disease-associated genes. Taking advantage of the new research tools and technologies, scientists are conducting genome-wide association studies to identify genetic differences between people with specific illnesses or conditions, and healthy individuals. Through this comparison, it has become possible to identify genetic differences that may affect whether an individual develops a particular disease.

Recent genome-wide association studies and other genetics studies have led to an explosion in the identification of genes and gene regions important in diseases within the NIDDK mission. Often the associated gene is unexpected—the function of the gene may be completely unknown or it may be involved in cellular processes that were not thought to be important in the particular disease. Some examples are highlighted in the chapters of this compendium, including genetic variants associated with type 1 diabetes, ulcerative colitis, gout, primary biliary cirrhosis, and hepatitis C.

Using genome-wide association studies, over 40 different regions of the genome have been identified that influence a person's risk of developing type 1 diabetes. Type 1 diabetes is an autoimmune disease in which the immune system launches a misguided attack and destroys the insulin-producing beta cells of the

pancreas. More information on type 1 diabetes and this study can be found in the Diabetes, Endocrinology, and Metabolic Diseases chapter.

Ulcerative colitis (UC) is a form of inflammatory bowel disease that causes inflammation in the tissues lining the colon and rectum. To expand knowledge of genetic contributors to UC, researchers performed a genome-wide association study using DNA collected from individuals with or without UC who shared a similar ancestry, in order to minimize other genetic differences. With this method, they were able to identify chromosomal regions, as well as genes within some of those regions, that are associated with an increased risk of developing UC. More information regarding this study can be found in the Digestive Diseases and Nutrition chapter.

Researchers have identified genetic variations in multiple regions of the genome that are associated with common measures of obesity that reflect total body fat and fat distribution. NIDDK-supported scientists carried out large-scale analyses of data from multiple genome-wide association studies. From this research, they uncovered genetic variations in six previously unreported regions (loci) of the genome that are associated with adult body mass index (a measure of weight adjusted to height). Read more about these findings in the Obesity chapter.

Under circumstances that are not yet completely understood, gout arises when excess uric acid begins to crystallize, causing inflammation and pain in the joints. Seeking to identify genes that contribute to increased uric acid levels and gout, researchers conducted genome-wide association studies. The Kidney, Urologic, and Hematologic Diseases chapter has information on the two new genes that were found to be associated with gout.

Available data suggest that there is a significant genetic predisposition to primary biliary cirrhosis—a chronic disease that causes the bile ducts in the liver to become inflamed and damaged and, ultimately, disappear. Genome-wide association studies were conducted to identify genetic loci associated with increased risk for this disease. The analysis identified several genetic variations in three specific genomic loci that are strongly associated with the patient group. To read more about the recently identified loci, see the Digestive Diseases and Nutrition chapter.

To discover gene variants associated with chronic kidney disease and kidney function, scientists performed genome-wide association scans on nearly 20,000 samples that had been collected during previous trials, and then replicated these results with a separate set of over 21,000 additional specimens. Several loci were found to be associated with kidney function, and one locus was linked to chronic kidney disease. More information on this study can be found in the Kidney, Urologic, and Hematologic Diseases chapter.

Hepatitis C is one of the major causes of chronic liver disease in the U.S. and a common cause of liver cancer. To identify host genetic factors that are required for successful hepatitis C infection, researchers utilized genome-wide scan technology. This approach confirmed the role of genetic factors previously implicated in hepatitis C infection and identified several host factors not previously suspected of playing a role in viral entry into the cell. See the Digestive Diseases and Nutrition chapter for more information.

With exciting genetic findings in hand, scientists can explore how these genes function in health and what goes wrong in disease. Research in this area may also set the stage for even more scientific breakthroughs on other disease-associated factors. For example, a newly associated gene may encode a protein that interacts with numerous other proteins. Therefore, discovering the disease association not only implicates the originally found gene and its encoded protein in the disease, but also the other proteins with which it interacts. This knowledge could illuminate several new therapeutic targets for disease prevention or treatment. Studying genes that were not thought to be involved in a disease can lead to new avenues for research that would likely not have been pursued otherwise. Identifying the functions of genes may not only enhance understanding of molecular mechanisms that underlie disease, but may also reveal new targets for diagnosis, risk assessment, and therapy.

AUTOIMMUNE DISEASES WITHIN THE NIDDK MISSION

This edition of *NIDDK Recent Advances & Emerging Opportunities* features research efforts that have revealed new information about several autoimmune diseases. Antibodies against one's own proteins

are termed "autoantibodies," and are a hallmark of autoimmune diseases. Scientists are learning more about the targets of the misguided immune attacks, and also are gaining insights into what goes awry in the immune system to unleash self-reactive antibodies. Brief snapshots are presented here, and more complete details of these research advances are found throughout this compendium.

Scientists discovered that in patients with autoimmune diseases such as type 1 diabetes or lupus, an immune system process called "receptor editing" is impaired, leading to the release of immune B cells that aberrantly produce autoantibodies. The immune system has several strategies for preventing the circulation of such destructive B cells throughout the body, one of which is "receptor editing," in which the DNA in the B cell is rearranged or shuffled to prevent the production of "self" antibodies. The finding that this process is impaired in patients with or at risk for autoimmune diseases could facilitate personalized treatments for these patients by helping to determine who might benefit from B cell therapies. For more information on this exciting research advance, see the write-up in the Diabetes, Endocrinology, and Metabolic Diseases chapter.

While studying the development of type 1 diabetes in an animal model, investigators identified a gene that may play a protective role in eliminating immune system cells that can destroy insulin-producing cells. The gene, called *Deaf1*, controls the production of molecules needed to eliminate self-reactive T cells, which are types of immune system cells. Cells in the pancreatic lymph nodes of mice make two forms of Deaf1 protein: a full-length, functional form and a shorter, variant form. Higher levels of the variant form were found to be associated with type 1 diabetes in mice and humans. See the Diabetes, Endocrinology, and Metabolic Diseases chapter for more on this intriguing finding and how it provides a potential target for drug development to treat the disease.

Researchers have recently reported how an immune system protein, called HLA-DQ8, contributes to increased immune reactivity towards dietary gluten in celiac disease. Celiac disease is an immune reaction to gluten, a protein found in wheat, rye, and barley. When people with celiac disease eat foods containing gluten, their immune system responds by damaging the small intestine—an autoimmune destructive condition. HLA proteins are found on the outer portion of cells, and the immune system uses this set of proteins to distinguish "self" cells from invaders. In people with celiac disease, HLA-DQ8 recognizes gluten fragments and presents them to the immune system, which results in a misguided inflammatory response. As described in the Digestive Diseases and Nutrition chapter, researchers show for the first time how the immune system recognizes different forms of gluten to generate an amplified immune response. Delineation of how the initial response develops paves the way for further investigations into how this response contributes to disease onset and progression.

New findings have recently been reported on the immunological events relevant to a debilitating autoimmune disease known as Goodpasture's syndrome (GPS). GPS is a rare condition marked by kidney damage, sometimes leading to kidney failure, and bleeding in the lungs. The underlying cause of GPS results from the body's own production of antibodies against a portion of the type IV collagen protein. As described in the Kidney, Urologic, and Hematologic Diseases chapter, investigators are beginning to uncover how immune B cells may generate antibodies to the body's own type IV collagen.

Researchers have identified a protein that may play a key role in the development of an autoimmune form of kidney disease known as "idiopathic membranous nephropathy." In this study, 70 percent of the tested blood samples from patients with idiopathic membranous nephropathy contained autoantibodies that attacked a single kidney protein; this kidney protein was ultimately identified as the M-type phospholipase A_2 receptor, or PLA_2R. The identification of the protein that induces the immune response will open new avenues of exploration in idiopathic membranous nephropathy. For additional information on this research advance, see the Kidney, Urologic, and Hematologic Diseases chapter.

PROTEIN FOLDING AND ENDOPLASMIC RETICULUM STRESS IN DISEASE

The correct three-dimensional structure of a protein is critical to its proper function. "Protein folding"—the

process by which a protein acquires its mature structure—occurs at several locations within the cell, including in the endoplasmic reticulum (ER). The ER performs several other functions, including additional processing of proteins, for example, to attach other necessary molecular components, and transporting proteins to the correct locations for them to perform their respective functions, whether within or outside the cell. These critical processes make the ER an important functional component of the healthy cell. Stress on the ER, whether caused by protein misfolding or other various stimuli, can lead to detrimental consequences, including cell death.

Protein misfolding can occur as a result of gene variations affecting the sequence of amino acids in a protein, or from defects in the folding process. Normally, misfolded proteins are eliminated by a cell's "quality control" system. However, in some cases, these misfolded proteins cannot be eliminated. Many diseases are associated with the aggregation of misfolded proteins, which can block cell function and induce cell death. Diseases also can result from the lack of properly folded proteins—without proper processing of these proteins, they are unable to function effectively. A number of diseases within the NIDDK's mission are known or suspected to be associated with protein misfolding and ER stress, including cystic fibrosis, alpha-1-anti-trypsin deficiency, polycystic kidney disease, type 2 diabetes, and possibly type 1 diabetes and inflammatory bowel disease.

To share information deriving from the study of diverse diseases and spur new research in this field, the NIDDK sponsored a workshop in January 2009 entitled *Protein Misfolding and Misprocessing in Disease*. Sessions of the workshop focused on the basic biology of protein folding and processing, insights gained from research on model organisms, as well as advances in understanding the biology of specific protein misfolding diseases, such as alpha-1-anti-trypsin deficiency, Wolfram syndrome, cystic fibrosis, and congenital hyperinsulinism. Other sessions focused the translation of these basic findings into novel therapeutic strategies, including the development and use of small molecule screens and other tools to identify potential therapeutics for treating a wide variety of diseases associated with protein misfolding.

In a recent advance highlighted in this compendium, scientists identified a factor that links ER stress with a condition called "lipotoxicity," which occurs when the amount of lipid exceeds the storage capacity of fat tissue, as is seen in obese patients. The identification of this factor helps explain how excess lipids can lead to cellular dysfunction, cell death, and organ failure, and may serve as a potential target for therapies directed toward combating obesity-related diseases.

In another advance described in this compendium, scientists discovered that chronic ER stress induced by a high-fat diet and obesity can lead to inappropriate glucose production in the liver. They found that the cell's response to ER stress and production of glucose during fasting shared a protein. When the cells are chronically stressed, this protein is stuck in one position, promoting the release of glucose, and possibly leading to insulin resistance.

Scientists also revealed that amyloids, highly packed protein structures commonly associated with diseases like Alzheimer's and type 2 diabetes, are not always the result of protein misfolding, as previously thought. Rather than a sign of dysfunction, these structures may, in some circumstances, serve an important role in storing hormones in a cell. However, in conditions such as a high-fat diet, stress, or older age, these structures may aggregate, harming the cells where they collect. This build-up of amyloids could explain, in part, the impaired function and loss of insulin-producing beta cells seen in type 2 diabetes.

Finally, scientists in NIDDK's Division of Intramural Research made ground-breaking technical advances to allow them to record how individual protein molecules fold into their correct biological shapes (see later in this chapter).

These exciting advances highlight important progress in understanding one of the biggest research questions in basic biology—protein folding and its role in health and disease. By studying the structural changes associated with going from an unfolded to a folded protein, scientists may discover new insights into the fundamental processes that guide protein folding and identify misfolding steps that can lead to disease. The NIDDK will continue to build on these discoveries and foster research to understand, prevent, and treat diseases

and conditions associated with protein misfolding and ER stress.

STUDIES IN PROTEIN FOLDING

Watching Proteins Take Shape One Molecule at a Time: Scientists in NIDDK's Division of Intramural Research have made ground breaking technical advances that allow them to study how individual protein molecules "fold" into their correct biological shapes. When a protein is made in a cell, it folds into a uniquely defined three-dimensional structure. This "native" structure, as it is called, is intimately linked to the protein's function, allowing the protein to properly perform chemical reactions, turn genes on or off, or interact with other proteins to regulate cellular communication. In cases where a protein's native structure is disrupted, such as by genetic mutations that alter its ability to fold properly, the resulting impairment of the protein's normal function can cause disease.

Given the importance of protein structure and function in health and disease, scientists have been interested in understanding the molecular determinants that lead to the formation of a protein's correct native structure. Traditional biophysical techniques have been used to uncover considerable knowledge about the structures of proteins in native and unfolded states and the factors that determine whether a protein folds or not. These techniques, however, have not allowed researchers to visualize the actual "path" or series of molecular events that takes a protein from the unfolded state to the native state. In a technical tour-de-force, NIDDK scientists have pushed the limits of a technique—called single-molecule fluorescence resonance energy transfer—to view, for the first time, the transitions between unfolded and native states during the folding process of individual protein molecules. By overcoming a number of technical issues that limited this technique, they were not only able to record discrete transitions between the unfolded and native states, but were also able to define an upper limit on the amount of time it takes for these transitions to occur. In addition to the study's technical achievement, the ability to observe individual folding transitions has important implications for the further study of protein folding. As all of the important structural changes associated with going from the unfolded state to the native state occur during these transitions, the application of this technique may allow researchers to uncover the fundamental mechanisms that guide the protein folding process and identify the misfolding steps that are often associated with diseases.

Chung HS, Louis JM, and Eaton WA: Experimental determination of upper bound for transition path times in protein folding from single-molecule photon-by-photon trajectories. Proc Natl Acad Sci USA 106: 11837-11844, 2009.

DELIVERING ANTI-INFLAMMATORY THERAPY

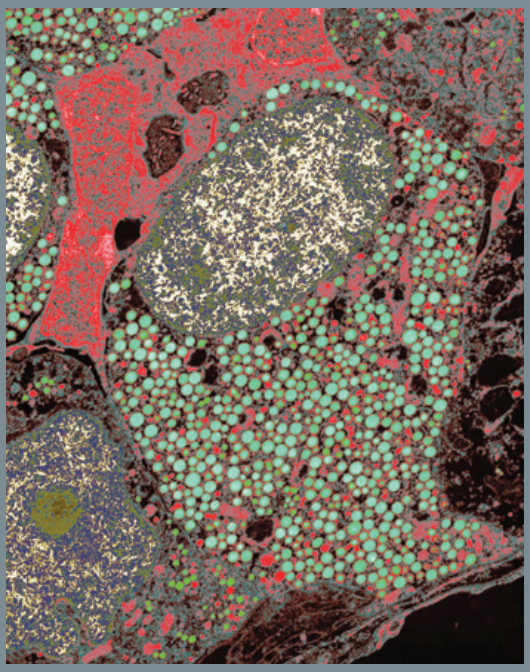
New Potential Therapeutic Strategy To Suppress Inflammation: Scientists have developed new technology with potential as a therapeutic strategy for inflammatory diseases by blocking production of proteins mediating inflammation. Inflammation is a key contributor to a variety of disorders including insulin resistance, cardiovascular disease, and autoimmune diseases. Short molecules of ribonucleic acid (RNA) can be targeted to reduce levels of specific proteins by interacting—or interfering—with the genetic material that encodes the protein, to prevent the protein from being made. This technique, known as "RNA interference," has potentially transformative therapeutic value. The development of a safe and effective way to deliver short interfering RNA molecules (siRNA) to specific types of cells *in vivo*, however, would be required before this technique could be used therapeutically. In a recent study, scientists designed a method to orally deliver siRNA to mouse macrophages, a cell type of the immune system that is important in initiating the inflammatory response. In this novel approach, layers of RNA molecules can be encapsulated within hollow, porous, tiny (micron-sized) shells of a substance called beta1,3-D-glucan, a non-toxic material made by yeast cells. The shells are recognized by proteins found primarily on the surface of macrophages, allowing for the specific uptake of the shells by macrophages. The scientists termed these shell particles "GeRPs" or glucan-encapsulated siRNA particles.

To test this system as a potential therapeutic in animals, the investigators examined the effects of feeding mice GeRPs with siRNA to a specific inflammatory protein known as Map4k4. They detected GeRPs inside

mouse macrophages in various tissues of the mouse body, including spleen, liver, and lungs, and observed a decrease in levels of Map4k4 in these tissues. The mice fed GeRPs with siRNA to Map4k4 were then given a toxic chemical that mimics a bacterial infection in order to stimulate the inflammatory response. When mice without the siRNA were given the chemical, their macrophages stimulated an excessive inflammatory response that was fatal to the animals. By feeding the mice siRNA to Map4k4, the scientists were able to halt the inflammatory response to the chemical, thus protecting the mice. This exciting result demonstrated that the orally administered siRNA was not only delivered to the correct cells—the macrophages—and carried to multiple tissues, but that the siRNA also reduced levels of Map4k4 and thus altered the mouse inflammatory response.

Inflammatory responses triggered by macrophages are involved in many conditions, including obesity, type 2 diabetes, inflammatory bowel disease, colitis, cardiovascular disease, atherosclerosis, and rheumatoid arthritis. The GeRP technology provides a novel oral delivery system for RNA interference to reduce levels of proteins involved in inflammation. Although further development and testing of the GeRP delivery system in animal models and in humans will be required, this study reveals the exciting potential of a new therapeutic strategy to suppress inflammation that may be applied to numerous adverse health conditions. In addition, this technology could be used to deliver siRNA, and possibly other cargo, to other types of cells in the immune system to alter their function and therefore could be explored as a potential therapy for autoimmune diseases like type 1 diabetes.

Aouadi M, Tesz GJ, Nicoloro SM, Wang M, Chouinard M, Soto E, Ostroff GR, and Czech MP: Orally delivered siRNA targeting macrophage Map4k4 suppresses systemic inflammation. Nature *458: 1180-1184, 2009.*



Research highlighted in this chapter describes a novel function for protein aggregates thought to be mainly associated with disease. Type 2 diabetes, like neurodegenerative diseases such as Alzheimer's disease, is associated with the appearance of amyloid protein in a patient's damaged tissues—the islet cells of the pancreas in diabetes (and the brain in Alzheimer's disease). Amyloids are defined by their structure—highly organized protein aggregates—rather than by the specific proteins that form them. Scientists supported by the NIDDK have discovered that amyloids can also have a normal biological function. In a recent study, they found that hormones can be stored as amyloids in an organized and concentrated form. This image illustrates the storage of hormones in granules (green) in a cell from the pituitary gland.

Image credit: University of Edinburgh/Wellcome Images.

Diabetes, Endocrinology, and Metabolic Diseases

NIDDK support of basic and clinical research in the areas of diabetes, endocrinology, and metabolic diseases spans a vast and diverse range of diseases and conditions, including diabetes, osteoporosis, cystic fibrosis, and obesity. Together, these diseases and conditions affect many millions of Americans and can profoundly decrease their quality of life. Many of them are complex—an interplay between genetic and environmental factors contributes to disease development.

Diabetes is a debilitating disease that affects an estimated 23.6 million people in the U.S.—or 7.8 percent of the total population—and is the seventh leading cause of death.[1] Diabetes lowers average life expectancy by up to 15 years,[2] increases cardiovascular disease risk two-to four-fold, and is the leading cause of kidney failure, lower limb amputations, and adult onset blindness.[1] In addition to these human costs, the estimated total financial cost for diabetes in the U.S. in 2007—including costs of medical care, disability, and premature death—was $174 billion.[1] Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.[1]

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone that is necessary for the body to absorb and use glucose (sugar) as a cellular fuel. These defects result in persistent elevation of blood glucose levels and other metabolic abnormalities, which in turn lead to the development of disease complications. The most common forms of diabetes are type 1 diabetes, in which the body loses its ability to produce insulin; and type 2 diabetes, in which the body becomes resistant to insulin signaling, with subsequent impaired insulin production.

Type 1 diabetes affects approximately 5 to 10 percent of individuals with diagnosed diabetes.[1] It most often develops during childhood, but may appear at any age. Type 1 diabetes is an autoimmune disease, in which the immune system launches a misguided attack and destroys the insulin-producing beta cells of the pancreas. If left untreated, type 1 diabetes results in death from starvation: without insulin, glucose is not transported from the bloodstream into the body's cells, where it is needed. Thus, patients require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—in order to regulate their blood glucose levels. Despite vigilance in disease management, with frequent finger sticks to test blood glucose levels and the administration of insulin, it is still impossible for patients to control blood glucose levels to near the normal levels achieved by functional beta cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery, as well as working to develop beta cell replacement therapies to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 95 percent of diabetes cases in the U.S.[1] Type 2 diabetes is associated with several factors, including older age and a family history of the disease. It is also strongly associated with obesity; more than 80 percent of adults with diabetes are overweight or obese.[3] Type 2 diabetes occurs at elevated rates among minority groups, including African Americans, Hispanic Americans, American Indians, and Native Hawaiians.[1]

In patients with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. As a result, blood glucose levels rise, and at first

[1] *The National Diabetes Fact Sheet. www.cdc.gov/diabetes/pubs/factsheet07.htm*

[2] *Portuese E and Orchard T: Mortality in Insulin-Dependent Diabetes. In* Diabetes in America *(pp. 221-232). Bethesda, MD: National Diabetes Data Group, NIH, 1995.*

[3] *Eberhardt MS, et al: MMWR 53: 1066-1068, 2004.*

the pancreas produces more insulin to compensate. Gradually, however, the pancreatic beta cells lose their capacity to secrete insulin, and the timing of insulin secretion becomes abnormal. Treatment approaches for controlling glucose levels include diet, exercise, and oral and injected medications, with insulin often required as the disease progresses. There are also an estimated 57 million adults in the U.S. who have a condition called "pre-diabetes," in which blood glucose levels are higher than normal, but not as high as in diabetes.[4] This population is at high risk of developing diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that patients with pre-diabetes can dramatically reduce their risk of developing full-blown diabetes with diet and exercise changes designed to achieve a 7 percent reduction in body weight.

Type 2 diabetes was previously called "adult-onset" diabetes because it is predominantly diagnosed in older individuals. However, this form of diabetes is increasingly being diagnosed in children and adolescents, and it disproportionately affects minority youth. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. First, the onset and severity of disease complications correlate with the duration of diabetes; thus, those with early disease onset are at greater risk with respect to complications than those who develop the disease later in life. Second, maternal diabetes during pregnancy—either onset of type 2 diabetes before pregnancy or the development of gestational diabetes during pregnancy—confers an increased risk of diabetes in offspring. Thus, the rising rates of diabetes and pre-diabetes in young women could lead to a vicious cycle of ever-growing rates of diabetes. Third, diabetes often becomes more difficult to control over time. With longer duration of disease, patients may find it increasingly difficult to strictly control their blood glucose levels and thus prevent or delay the development of complications. Therefore, the advent of type 2 diabetes in youth has the potential to worsen the enormous health burden that diabetes already places on the U.S. New research described in this chapter helps define the scope of the pediatric and maternal diabetes problem, and underscores the need to address this health challenge.

The NIDDK is supporting research to better understand metabolism, and the mechanisms that lead to the development and progression of diabetes and the many other endocrine and metabolic diseases within the Institute's mission; such research will ultimately spur the design of potential new intervention strategies. In parallel, based on knowledge from past scientific research investments, the Institute is vigorously pursuing studies of prevention and treatment approaches for these diseases.

GENETICS OF DIABETES

Unraveling the Genetic Causes of Type 1 Diabetes: Several recent studies are contributing to understanding the genetic underpinnings of type 1 diabetes. Scientists in the NIDDK-supported Type 1 Diabetes Genetics Consortium (T1DGC) studied over 2,400 families and discovered that variants in the *UBASH3A* genetic region were associated with the disease. They also confirmed previously reported associations with three other genetic regions (*INS*, *IFIH1*, and *KIAA0305*). A study by a different group of scientists analyzed several patient populations to follow up on results of recent genome-wide association studies (GWAS). These populations included people enrolled in the T1DGC, as well as participants from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study (DCCT/EDIC), which is a long-term NIDDK-supported study of people with type 1 diabetes. These researchers also identified *UBASH3A* as being associated with type 1 diabetes and, in addition, discovered an association in the *BACH2* genetic region. The UBASH3A protein is predominantly found in immune system cells called T cells, and *BACH2* is thought to be a regulator of the immune system's antibody response. Because type 1 diabetes is an autoimmune disease, it is plausible that defects in these genes could contribute to type 1 diabetes, although more research will help determine how these genes may play a role. In another study, scientists examined a different patient population to confirm the association between variants in the *IFIH1* genetic region and type 1 diabetes. The *IFIH1* gene was found to be more strongly expressed (turned on) in some immune system cells from people carrying variants of the gene associated with type 1 diabetes. This observation suggests that higher amounts of the protein encoded

[4] *http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm*

by the *IFIH1* gene may be linked to increased risk for type 1 diabetes, but additional studies in more people are necessary to confirm the finding. *IFIH1* is also thought to play a role in the immune system and has been linked to two other autoimmune diseases. In another study, T1DGC scientists combined data from a new GWAS with data from previous studies to discover that over 40 different genetic regions influence a person's risk of developing type 1 diabetes. That number includes the genetic regions described above, as well as several novel regions.

Scientists are also building on recent genetics findings to understand how other genetic factors contribute to risk for type 1 diabetes. Researchers looked at previously identified type 1 diabetes susceptibility genes to determine their impact on an early stage in type 1 diabetes onset—the development of autoimmunity—in children participating in the NIDDK-supported Diabetes Autoimmunity Study in the Young (DAISY). These children were originally enrolled in DAISY because they carry variants for a different diabetes susceptibility gene (*HLA*) that put them at high genetic risk for developing type 1 diabetes. The scientists discovered that a variant in the *PTPN22* gene region increased the risk of developing autoimmunity in children with a family history of the disease. In contrast, a variant of the *CTLA-4* gene region increased the risk of autoimmunity in children without such a family history.

These studies are shedding new light on genetic factors that underlie type 1 diabetes, and may lead to enhanced ways to predict who is at high-risk for the disease, and potentially inform new intervention approaches. They also demonstrate how new knowledge is stemming from long-term, NIDDK-supported research studies based on new and emerging genetics technologies.

Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, and Rich SS; The Type 1 Diabetes Genetics Consortium: Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet 41: 703-707, 2009.

Concannon P, Onengut-Gumuscu S, Todd JA, Smyth DJ, Pociot F, Bergholdt R, Akolkar B, Erlich HA, Hilner JE, Julier C, Morahan G, Nerup J, Nierras CR, Chen WM, and Rich SS; Type 1 Diabetes Genetics Consortium: A human type 1 diabetes susceptibility locus maps to chromosome 21q22.3. Diabetes 57: 2858-2861, 2008.

Grant SF, Qu HQ, Bradfield JP, Marchand L, Kim CE, Glessner JT, Grabs R, Taback SP, Frackelton EC, Eckert AW, Annaiah K, Lawson ML, Otieno FG, Santa E, Shaner JL, Smith RM, Skraban R, Imielinski M, Chiavacci RM, Grundmeier RW, Stanley CA, Kirsch SE, Waggott D, Paterson AD, Monos DS; DCCT/EDIC Research Group, Polychronakos C, and Hakonarson H: Follow-up analysis of genome-wide association data identifies novel loci for type 1 diabetes. Diabetes 58: 290-295, 2009.

Liu S, Wang H, Jin Y, Podolsky R, Reddy MV, Pedersen J, Bode B, Reed J, Steed D, Anderson S, Yang P, Muir A, Steed L, Hopkins D, Huang Y, Purohit S, Wang CY, Steck AK, Montemari A, Eisenbarth G, Rewers M, and She JX: IFIH1 *polymorphisms are significantly associated with type 1 diabetes and* IFIH1 *gene expression in peripheral blood mononuclear cells. Hum Mol Genet 18: 358-365, 2009.*

Steck AK, Zhang W, Bugawan TL, Barriga KJ, Blair A, Erlich HA, Eisenbarth GS, Norris JM, and Rewers MJ: Do non-HLA genes influence development of persistent islet autoimmunity and type 1 diabetes in children with high-risk HLA-DR,DQ *genotypes? Diabetes 58: 1028-1033, 2009.*

Little Genetic Overlap Between Type 1 and Type 2 Diabetes: Research has shown that there is little genetic association between the two major forms of diabetes. Because of increasing recognition that some people have clinical features of both type 1 and type 2 diabetes, scientists sought to determine whether the two diseases had common genetic underpinnings by examining whether 12 recently-identified type 2 diabetes gene regions were also involved in type 1 diabetes. (A previous study had shown that a set of genes affecting risk for type 1 diabetes does not affect the risk of type 2 diabetes.) The new study found a possible association with only one gene, called *PPARG*, which is known to play a role in type 2 diabetes. The PPARG protein is thought to have a role in the immune system, which might help explain its possible involvement in type 1 diabetes, an autoimmune disease. The overall lack of genetic overlap between the two major forms of diabetes reinforces the notion that type 1 and type 2 diabetes result from distinct physiological processes.

Raj SM, Howson JM, Walker NM, Cooper JD, Smyth DJ, Field SF, Stevens HE, and Todd JA: No association of multiple type 2 diabetes loci with type 1 diabetes. Diabetologia 52: 2109-2116, 2009.

PANCREATIC PROGENITOR CELLS

New Insights into Pancreatic Development: New studies are providing key insights into the progenitor cells that give rise to different cell types in the pancreas. Both type 1 and type 2 diabetes are characterized by loss of functional beta cells, the pancreatic cells that produce the hormone insulin. Strategies to repopulate beta cells, either by transplant or by induction of new beta cell formation, show promise in treating diabetes. Toward successful therapeutic strategies, scientists are making progress in identifying, characterizing, and understanding the factors and mechanisms that underlie pancreatic development. In addition to the insulin-producing beta cells, the pancreas is composed of multiple other cell types. Some of these—like beta cells—produce hormones released into the blood to regulate the body's metabolism; these cell types are termed "endocrine." Other cell types produce proteins that aid in the digestion of food; these cell types are termed "exocrine." In order to promote the formation of new beta cells, scientists, including members of the NIDDK-supported Beta Cell Biology Consortium, are determining when and how certain pancreatic progenitor cells become "committed" to developing into specific endocrine or exocrine cell types.

In one study, scientists investigated the role of a group of proteins, called presenilins, in specifying pancreatic cell types in mice. They studied cells that have Ngn3, a well-established marker of embryonic pancreatic progenitor cells, and discovered that the activity of presenilins was needed to block the cells from becoming exocrine cells. This showed that Ngn3 was not sufficient to commit pancreatic progenitor cells to endocrine cell types. Rather, cells that have Ngn3 can become endocrine or exocrine cells—a flexibility of Ngn3 cells that was previously unknown. In a second study, using elegant labeling techniques to mark a single mouse pancreatic progenitor cell and monitor its progression, another group of scientists focused on the Ngn3 cells that become endocrine cells and found that these cells are "unipotent" precursors. That is, Ngn3 is a marker shared in common by cells destined to develop into different endocrine cell types, but each individual Ngn3-containing cell is committed to becoming only one of the endocrine cell types. In fact, unlike other progenitor cells that proliferate to generate many mature cells, Ngn3-containing cells often appeared not to proliferate at all, with each one simply morphing into its final endocrine cell type. These findings are important because understanding the characteristics of progenitor cells that can turn into beta cells can help inform new strategies toward generating new beta cells to replace those impaired by diabetes.

In a final study, scientists uncovered additional plasticity in a pancreatic endocrine cell type—the alpha cell. Using genetic techniques in mice, the researchers increased the levels of a protein called Pax4, which is known to be involved in promoting cells to develop into endocrine cell types. They found that mice with high levels of Pax4 had oversized clusters of beta cells, which resulted from alpha-beta precursor cells and established alpha cells being induced to form beta cells. In addition, in a mouse model of diabetes, the high levels of Pax4 promoted generation of new beta cells and overcame the diabetic state. The discovery that alpha cells have the potential to convert to beta cells, and the additional insights into pancreatic progenitor cells made in the other studies, generate a fuller picture of pancreatic development. These advances may pave the way toward new cell-based therapies for diabetes.

Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N, Madsen OD, Serup P, Heimberg H, and Mansouri A: The ectopic expression of Pax4 *in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell 138: 449-462, 2009.*

Cras-Méneur C, Li L, Kopan R, and Permutt MA: Presenilins, Notch dose control the fate of pancreatic endocrine progenitors during a narrow developmental window. Genes Dev 23: 2088-2101, 2009.

Desgraz R and Herrera PL: Pancreatic neurogenin 3-expressing cells are unipotent islet precursors. Development 136: 3567-3574, 2009.

AUTOIMMUNITY IN TYPE 1 DIABETES

***Deaf1* Gene May Play a Role in Type 1 Diabetes:** Scientists identified a gene that may play a role in the

development of type 1 diabetes. Examining genes from a mouse model of type 1 diabetes, the scientists found that cells in the animals' pancreatic lymph nodes make two forms of the same gene called deformed epidermal autoregulatory factor 1 (*Deaf1*). One form of this gene encodes full-length, functional Deaf1 protein, while the other encodes a shorter, nonfunctional variant form. The research suggests that the full-length, functional form of Deaf1 may control the production of molecules needed to eliminate immune cells that can destroy insulin-producing cells in the pancreas, causing type 1 diabetes. Moreover, the presence of the Deaf1 variant was found to prevent the full-length Deaf1 protein from functioning normally. Additional experiments showed that the variant form inhibited turning on genes needed to produce certain molecules involved in immune regulation. Researchers also found that levels of the variant form of Deaf1 were higher in people with type 1 diabetes compared to levels in people without the disease. In addition, the human variant form inhibited the full-length form from functioning normally. The research suggests that the development of type 1 diabetes may in part be due to increased levels of the Deaf1 variant protein in pancreatic lymph nodes. Increased levels of Deaf1 variant may, in turn, lead to reduced production of molecules that are required to educate the immune system not to attack the body's own cells, including the insulin-producing cells of the pancreas. Furthermore, the findings suggest that the Deaf1 variant form may predict risk for type 1 diabetes and be a target for therapy.

Yip L, Su L, Sheng D, Chang P, Atkinson M, Czesak M, Albert PR, Collier AR, Turley SJ, Fathman CG, and Creusot RJ: Deaf1 isoforms control the expression of genes encoding peripheral tissue antigens in the pancreatic lymph nodes during type 1 diabetes. Nat Immunol 10: 1026-1033, 2009.

B Lymphocytes Do the Shuffle: "Receptor Editing" as a Marker for Type 1 Diabetes: Scientists discovered that patients with autoimmune diseases have lower levels of "receptor editing" in the B lymphocytes of their immune systems than healthy individuals—a discovery that may lead to changes in strategies for treatment of some patients with type 1 diabetes. In mammals, B cells generate diverse antibodies to recognize a variety of potential foreign invaders, such as viruses or bacteria. The DNA that encodes an antibody undergoes rearrangements to create antibodies that can recognize different substances. Sometimes an errant B cell produces an antibody that recognizes "self"—the body's own cells. As one way to correct the error, the DNA in the B cell can be shuffled again, a process called "receptor editing."

In patients with autoimmune diseases, however, the process of receptor editing seems to falter. In this study, scientists designed a new assay to investigate the timing and incidence of editing during B cell development by measuring the occurrence of rearrangement with a non-functional segment of antibody DNA. Other assays of autoimmunity rely on measurements of the antibodies themselves once a B cell has matured (a late event). This new assay allows measurement of earlier events potentially associated with autoimmunity, as editing occurs during B cell development in the bone marrow while the cell is still maturing. Understanding the timing of this process could provide insight about whether or how to direct B cell-targeted therapies. To investigate the level of receptor editing in autoimmune diseases, the scientists first conducted their assay with mouse models of type 1 diabetes and systemic lupus erythematosus. Mice that had already developed autoimmunity showed a reduced level of editing, indicating that reduced editing is associated with autoimmune disease. In addition, in further experiments the scientists observed that mice prone to autoimmunity showed lower levels of editing even before autoimmune disease developed; this result suggests that reduced editing could indicate a predisposition towards the development of autoimmunity rather than arising as a consequence.

To determine whether this association was also found in people, the researchers examined B cells from adult patients with type 1 diabetes or systemic lupus erythematosus. They found that some of the patients had low levels of receptor editing when compared to healthy individuals. This indicates that the low levels may be correlated with these diseases, but that not all autoimmunity results from reduced receptor editing. Critically, these results suggest that current therapies to temporarily delete mature B cells may not be effective in patients with reduced receptor editing, because new B cells that recognize "self" will continue to be generated in the bone marrow, unimpeded by editing. This new assay to measure receptor editing could facilitate personalized

treatments for patients with type 1 diabetes or lupus by helping to determine who may benefit from B cell therapies. Technical features of the assay additionally make it potentially applicable to other autoimmune diseases. Further research will show whether the assay may also provide an early indication of increased risk for developing autoimmunity.

Panigrahi AK, Goodman NG, Eisenberg RA, Rickels MR, Naji A, and Luning Prak ET: RS rearrangement frequency as a marker of receptor editing in lupus and type 1 diabetes. J Exp Med 205: 2985-2994, 2008.

NIDDK Director Testifies on *Special Diabetes Program*

On June 24, 2009, NIDDK Director Dr. Griffin P. Rodgers testified about progress in type 1 diabetes research before the Senate Committee on Homeland Security and Governmental Affairs. The hearing, entitled "Type 1 Diabetes Research: Real Progress and Real Hope for a Cure," was chaired by Senators Joe Lieberman and Susan Collins. Dr. Rodgers spoke of research made possible by the *Special Statutory Funding Program for Type 1 Diabetes Research*, including the discovery of at least 40 genetic regions linked to type 1 diabetes, and progress from clinical trials testing approaches to delay or prevent the disease. A hearing on type 1 diabetes research is held every 2 years in conjunction with the Juvenile Diabetes Research Foundation (JDRF) Children's Congress. The previous day, Dr. Rodgers received the JDRF Children's Congress Heroes Award for his work in advancing type 1 diabetes research and improving the lives of people affected by the disease.

At the June 2009 congressional hearing on type 1 diabetes, JDRF Children's Congress delegates (foreground) listened to testimony from (at table, left to right) JDRF International Chair Mary Tyler Moore, NIDDK Director Dr. Griffin Rodgers, boxing legend Sugar Ray Leonard, and singer/songwriter Nick Jonas. Several of the children and a parent also spoke at the hearing, describing their experiences with this disease and the importance of research.

RISK FACTORS FOR DIABETES IN YOUTH

Height Growth Rate Is Associated with Type 1 Diabetes Development in At-Risk Children:

Researchers discovered an association between progression to type 1 diabetes in children and an accelerated rate of height growth (change in height over time). Type 1 diabetes results from a complex interplay of both genetic and environmental causes. Since 1993, the Diabetes Autoimmunity Study in the Young (DAISY) has followed children at increased type 1 diabetes genetic risk for the development of autoimmunity—a pre-clinical phase that often precedes the clinical diagnosis—and type 1 diabetes. Following these high-risk children for many years allows the scientists to note changes as the children age and to monitor who develops the disease and who does not.

In one recent analysis of data collected from the DAISY study, the scientists compared height, weight, body mass index (BMI, a measure of weight relative to height)—and the rates of change of these characteristics—between children who developed autoimmunity and type 1 diabetes and children who did not. They found that, in children ages 2 to 11 and genetically prone to the disease, a greater rate of height growth was associated with the development of autoimmunity and strongly associated with progression to type 1 diabetes in children with autoimmunity. They did not observe a strong correlation between autoimmunity or type 1 diabetes development and final height, weight, BMI, or growth rate of weight or BMI. Previous studies suggested an association between increasing BMI, weight, and height and incidence of type 1 diabetes in the general population, but this study was specific to genetically at-risk children ages 2 to 11. Understanding how more rapid height change could trigger autoimmunity and type 1 diabetes may help to elucidate how the disease develops. For example, rapid growth might stress the insulin-producing beta cell. Additional research could confirm and determine the reason for the association between increased height growth rate and type 1 diabetes development.

Lamb MM, Yin X, Zerbe GO, Klingensmith GJ, Dabelea D, Fingerlin TE, Rewers M, and Norris JM: Height growth velocity, islet autoimmunity and type 1 diabetes development: the Diabetes Autoimmunity Study in the Young. Diabetologia 52: 2064-2071, 2009.

Studies Highlight Health Disparity in Risk Factors for Type 2 Diabetes in Middle School Children: Researchers have found a high level of risk for future diabetes and cardiovascular disease among middle school students. Scientists recruiting sixth-grade students for a new intervention study found that minority students were at greater risk for type 2 diabetes than Caucasians. HEALTHY is a 3-year intervention study designed to test approaches for reducing risk factors for type 2 diabetes in middle school children. The intervention involves making changes to the school environment: improving nutrition, increasing the students' physical activity, and helping the students and their families make behavior changes. Prior to the start of the intervention, the researchers collected blood samples and other information from the students to examine the prevalence of type 2 diabetes risk factors for this population of children. Over 6,000 sixth-grade students attending 42 schools in the U.S. were included in the survey. At every school, at least half of the students were eligible for free or reduced-priced lunch or belonged to a minority group. Investigators measured three different characteristics in students—body mass index (BMI, a measure of weight relative to height), fasting blood glucose levels, and fasting insulin levels—to determine if they had risk factors for type 2 diabetes. A high BMI and elevated fasting blood glucose and insulin levels have been linked to development of type 2 diabetes. The researchers recently reported their findings.

Overall, nearly half of the sixth-grade students in schools participating in the HEALTHY study were considered overweight or obese according to their BMI. This is higher than the national average of U.S. children, but similar to rates observed in other predominantly minority populations. Among the students in the study, the Hispanic children had the greatest percentage of overweight/obese individuals, followed by African American children. In addition to measuring BMI, investigators also determined the fasting blood glucose and fasting insulin levels of the students. Sixteen percent of the students had relatively high levels of blood glucose while fasting (pre-diabetes), and almost 7 percent had elevated fasting insulin levels. Similar to the findings on BMI, the highest percentage of pre-diabetes and elevated fasting insulin levels was observed in Hispanic students. Together, these results indicate the importance of research focusing on children, particularly those in populations similar to those in the HEALTHY study, to test interventions to reduce the risk of type 2 diabetes with the hope of achieving long-lasting improvements to children's health.

Another study examined a racially and ethnically diverse group of eighth-grade students participating in a pilot study for HEALTHY to see what fraction met the International Diabetes Federation (IDF) metabolic syndrome definition. The "metabolic syndrome" refers to a group of risk factors that increase the likelihood of developing cardiovascular disease and type 2 diabetes. Standard definitions of the metabolic syndrome apply only to adults.

Recently, the IDF developed a definition for children. According to IDF criteria, an adolescent has metabolic syndrome if he or she has a large waist circumference (≥90th percentile) and two or more of the following: high blood triglycerides (a type of fat that increases risk for heart disease); low HDL ("good cholesterol"); high blood pressure; or elevated fasting blood glucose.

The researchers found that, overall, 9.5 percent of the children in the HEALTHY pilot study met the IDF pediatric definition for metabolic syndrome—double the rate previously found among American teens in the general population. More than 80 percent of the children included in the analysis were Hispanic or African American—groups at an increased risk of type 2 diabetes. Moreover, 50 percent of the children were overweight. This result, along with the results from HEALTHY, highlights a disparity in disease risk among adolescent Hispanic and African American children and underscores the importance of diabetes and cardiovascular disease prevention efforts to address health disparities among children.

Kaufman FR, Hirst K, Linder B, Baranowski T, Cooper DM, Foster GD, Goldberg L, Harrell JS, Marcus MD, and Treviño RP, for the HEALTHY Study Group: Risk factors for type 2 diabetes in a sixth-grade multiracial cohort: the HEALTHY study. Diabetes Care 32: 953-955, 2009.

Jago R, Baranowski T, Buse J, Edelstein S, Galassetti P, Harrell J, Kaufman F, Linder B, and Pham T, for the Studies to Treat or Prevent Pediatric Type 2 Diabetes Prevention Study Group: Prevalence of the metabolic syndrome among a racially/ethnically diverse group of U.S. eighth-grade adolescents and associations with fasting insulin and homeostasis model

assessment of insulin resistance levels. Diabetes Care 31: 2020-2025, 2008.

Maternal Diabetes Accelerates Type 2 Diabetes Diagnosis in Offspring: A surveillance study of diabetes in youth has found that children with type 2 diabetes received their diagnosis at an earlier age if their mothers had been diagnosed with diabetes prior to pregnancy—adding to the body of evidence about the long-term effects of intrauterine exposure to diabetes on offspring. These results come from the SEARCH for Diabetes in Youth Study. SEARCH is a large, population-based study of diabetes in racially and ethnically diverse youth.

To learn more about why higher rates of type 2 diabetes in children are associated with maternal diabetes, SEARCH investigators compared the parental diabetes history of over 2,600 youth diagnosed with either type 1 or type 2 diabetes before age 20. They found that not only were youth with type 2 diabetes nearly eight times more likely than those with type 1 diabetes to have a mother with diabetes, but they were also more likely to have been diagnosed more than 18 months sooner, on average, if their mothers already had diabetes while pregnant than if they didn't. These findings held for all ethnic and racial groups studied. The results of this study build on earlier studies of diabetes in youth among the Pima Indians of Arizona, by showing that the increased risk of diabetes conferred by intrauterine exposure to diabetes is not restricted to this population. Moreover, SEARCH found that, among the children with type 2 diabetes whose mothers also had diabetes, girls had an earlier age of diagnosis than boys by over a year on average. These findings are especially troubling in light of the cycle in which earlier onset of type 2 diabetes leads to greater likelihood of maternal type 2 diabetes and in turn increased risk in offspring, and lend urgency to efforts to break this cycle and thus reduce one of the risk factors for diabetes in youth.

Pettitt DJ, Lawrence JM, Beyer J, Hillier TA, Liese AD, Mayer-Davis B, Loots B, Imperatore G, Liu L, Dolan LM, Linder B, and Dabelea D: Association between maternal diabetes in utero and age at offspring's diagnosis of type 2 diabetes. Diabetes Care 31: 2126-2130, 2008.

Prevalence of Overweight and Obesity in Young Americans with Diabetes: In recent decades, overweight and obesity rates in children have risen dramatically in the U.S., with a disproportionate impact on minority youth. Obesity puts young people at risk of developing other conditions, such as diabetes. While the relationship between obesity and type 2 diabetes is well known, less is understood about how overweight or obesity affects type 1 diabetes development.

To improve understanding of the degree to which increased body fat contributes to the risk of diabetes among American youth with either form of diabetes, researchers determined the prevalence of overweight and obesity in young Americans with type 1 or type 2 diabetes, compared to those without diabetes. To do so, they utilized data collected from 2001 to 2004 as part of a large, multi-center, population-based study with diverse racial and ethnic representation, called the SEARCH for Diabetes in Youth study (SEARCH). Results of this study were compared with information collected during the same timeframe on young Americans without diabetes who participated in the National Health and Nutrition Examination Survey (NHANES), led by the Centers for Disease Control and Prevention (CDC). Scientists analyzed and compared data collected from Americans younger than 20 years old in both these studies to assess the contribution of overweight or obesity, based on the body mass index (a measure of weight relative to height), to a medical diagnosis of type 1 or type 2 diabetes. As anticipated, a majority (79.4 percent) of the children and adolescents with type 2 diabetes were obese and an additional 10.4 percent were overweight. Overall, young people with type 1 diabetes were more likely to be overweight (22 percent vs. 16 percent), but not obese, compared to youth who did not have diabetes. Researchers could not determine whether the increase in overweight preceded the diagnosis of type 1 diabetes or was caused by its therapy. Intensive therapy with insulin to control glucose levels and reduce diabetes complications is associated with an increased risk of overweight.

As the largest racially and ethnically diverse study to date focused on the prevalence of overweight and obesity in American children and adolescents with diabetes, this study provides information that is critical to understanding and addressing this issue. By showing an association between excess weight and type 1 diabetes, as well as supporting earlier findings of the link between

obesity and type 2 diabetes, this new study highlights areas for future investigation, aimed at improving care for these chronic conditions in young people in the U.S.

Liu LL, Lawrence JM, Davis C, Liese AD, Pettitt DJ, Pihoker C, Dabelea D, Hamman R, Waitzfelder B, and Kahn HS for the SEARCH for Diabetes in Youth Study Group: Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for Diabetes in Youth Study. Pediatr Diabetes 2009 May 15. [Epub ahead of print]

DIABETES AND HEART DISEASE

Treating Heart Disease in People Who Have Diabetes: New results from a randomized clinical trial in nearly 2,400 patients indicate that optimal medical therapy is as beneficial as elective revascularization procedures in patients with type 2 diabetes and stable coronary heart disease. Type 2 diabetes more than doubles the risk of heart attack and stroke and also worsens outcomes after these events. While revascularization (*e.g.*, coronary bypass surgery or angioplasty) has proven beneficial in treating severe forms of coronary artery disease, its benefits for people with diabetes and stable coronary artery disease have been uncertain.

Led by the National Heart, Lung, and Blood Institute with support from NIDDK, the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) multi-center, international clinical trial simultaneously compared two cardiovascular treatment approaches—revascularization procedures and optimal medical therapy—and two diabetes control strategies, in an effort to identify ways to improve patient survival and to lower the risk of heart attacks and strokes. Optimal medical therapy includes intensive drug therapy and lifestyle interventions, such as dietary changes and smoking cessation. After an average patient follow-up of 5 years, BARI 2D found no overall difference between revascularization procedures and medical therapy in lowering the risk of death, heart attack, and stroke. The researchers did see an intriguing difference among patients within the revascularization group. Although the study was not designed to compare the efficacy of different forms of revascularization in patients for whom this is an elective procedure, researchers observed that patients who had prompt bypass surgery, rather than angioplasty, as their form of revascularization, had significantly fewer non-fatal heart attacks or strokes compared to similar patients who initially received optimal medical therapy alone. However, participants who were treated with bypass surgery, and their counterparts in the medical therapy group, were also more likely to have had more extensive coronary artery disease when they entered the trial than those who were treated with angioplasty—possibly explaining the relative difference in outcome with the two revascularization procedures. More research is needed to confirm the findings about bypass surgery in type 2 diabetes patients for whom this is an elective procedure. Researchers also found no difference between the two diabetes control strategies tested in the trial. Because heart disease is the leading cause of death in people with diabetes, findings such as these are important to help inform treatment choices by patients and health care providers.

Frye RL, August P, Brooks MM, Hardison RM, Kelsey SF, MacGregor JM, Orchard TJ, Chaitman BR, Genuth SM, Goldberg SH, Hlatky MA, Jones TLZ, Molitch ME, Nesto RW, Sako EY, and Sobel BE, for the BARI 2D Study Group: A randomized trial of therapies for type 2 diabetes and coronary artery disease. N Engl J Med 360: 2503-2515, 2009.

Understanding Heart Health in Youth with Type 1 Diabetes: New research by the SEARCH for Diabetes in Youth study showed that youth with type 1 diabetes and suboptimal control of their blood glucose levels had abnormal lipid (fat) profiles, even after a short duration of disease. Diabetes is a major risk factor for heart disease, but most studies on the link between the two diseases have been done in adults. To determine if lipid abnormalities appear at young ages, SEARCH investigators compared lipid profiles in youth with and without type 1 diabetes, and examined if variations in lipid profiles were associated with differences in blood glucose control. Lipid profiles, such as measurements of total cholesterol and LDL ("bad") cholesterol, are related to risk of heart disease in adults. The youth in the study were 10 to 22 years old and had had type 1 diabetes for an average of about 4 years. The scientists found that youth with type 1 diabetes and optimal blood glucose control (HbA1c less than 7.5 percent) had similar lipid levels as non-diabetic youth. However, youth with type 1 diabetes and suboptimal glucose

control (HbA1c of 7.5 percent or greater) had elevated lipid levels, including high total and LDL cholesterol. Regardless of blood glucose control, youth with the disease had elevated apolipoprotein B levels and had more small, dense LDL particles (two types of lipids associated with heart disease risk in adults) compared to non-diabetic youth. These results suggest that youth with type 1 diabetes have abnormal lipid levels, even after a relatively short duration of disease. However, good blood glucose control may help protect against these abnormalities, which provides further impetus for people with type 1 diabetes to implement early and intensive blood glucose control.

Guy J, Ogden L, Wadwa RP, Hamman RF, Mayer-Davis EJ, Liese AD, D'Agostino R Jr, Marcovina S, and Dabelea D: Lipid and lipoprotein profiles in youth with and without type 1 diabetes: the SEARCH for Diabetes in Youth case-control study. Diabetes Care 32: 416-420, 2009.

REGULATORS OF METABOLISM IN HEALTH AND DISEASE

Insulin, Metformin, and Pathways of Glucose Production in Fasting and Obesity: New research is shedding light on the ways metabolism in the liver is affected by obesity and by two of the most widely prescribed medications for people with diabetes. Insulin, produced naturally by the body in response to elevated glucose in the blood, is prescribed to all patients with type 1 diabetes because they cannot make the vital hormone themselves. It is also prescribed to many people with type 2 diabetes in cases where other medications cannot make up for lost insulin production capacity and their bodies' increased needs for the hormone. The most widely prescribed medication for type 2 diabetes, however, is metformin, which works by reducing the amount of glucose fed into the bloodstream by the liver. A hormone called glucagon triggers the liver to release glucose during periods of fasting. For reasons that have not been fully understood, liver glucose production occurs even in the absence of fasting in people with diabetes, contributing to elevated blood glucose. One of insulin's key effects is to blunt the impact of glucagon, stopping its release of liver glucose, and accounting for one of its most serious side effects. Overly high doses of insulin not only send glucose levels too low, they also limit the ability of glucagon to bring glucose back up again. The result is hypoglycemia, dangerously low blood glucose. Metformin also counteracts glucagon, but rarely causes hypoglycemia by itself, because it does not directly lower blood glucose by signaling cells to take up glucose as insulin does.

New research in obese mice fed a high-fat diet clarifies the pathway that leads to excessive production of glucose from the liver in type 2 diabetes, and pinpoints the ways in which metformin and insulin interrupt the process. One group of researchers found that a complex of proteins acts to boost glucose output and is triggered both by fasting signals (glucagon) and by a cellular condition that can result from obesity, called the "ER (endoplasmic reticulum) stress response" (see Cross-Cutting Science chapter). Another group of researchers found that both insulin and metformin lead to a modification of one of the proteins in this complex—a protein called CBP. The modification of CBP causes the complex to fall apart so that it no longer supports glucose production. Although the impact on CBP and glucose production is the same, metformin and insulin work through different pathways to modify CBP, which helps explain why metformin is effective even in patients who are resistant to insulin's effects. Understanding the molecular pathways by which the healthy liver promotes glucose control, as well as how insulin and metformin work in disease, has the potential to help improve glucose control in diabetes patients, preventing both hypoglycemia and the long-term complications of hyperglycemia (high blood glucose). Because metformin is currently the only approved drug in its class, this research may also help to identify new and better therapeutic strategies to help people with diabetes control their blood glucose.

He L, Sabet A, Djedjos S, Miller R, Sun X, Hussain MA, Radovick S, and Wondisford FE: Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. Cell 137: 635-646, 2009.

Wang Y, Vera L, Fischer WH, and Montminy M: The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. Nature 460: 534-537, 2009.

A Metabolic Sensor Controls Energy Balance, Inflammation, and Insulin Resistance: Scientists have discovered how a key regulatory protein controls metabolic function and "energy balance"—the balance between energy (in the form of calories) ingested and energy burning or storage. The proper balance of energy intake, storage, and usage is a fundamental aspect of human health, as the disruption of the energy balance equation often leads to metabolic disorders such as type 2 diabetes, metabolic syndrome, and obesity. Regulation of the body's energy balance involves an extensive and intricate network of cellular metabolic pathways. At the center of this network is a protein called SIRT1. Acting as a "metabolic sensor," SIRT1 directs cells to turn on or off different pathways that either use or store energy in response to the energy needs of the cell. In two new studies, scientists have uncovered how SIRT1 coordinates with different pathways in different tissues to influence metabolic function.

In the first study, researchers found that SIRT1 coordinates with another key regulator of cellular energetics, the AMP-activated protein kinase (AMPK). AMPK senses and responds to levels of energy deprivation, such as during fasting or starvation, and directs cells to start breaking down stores of fat. To do this, AMPK activates a protein known as PGC-1alpha, which subsequently turns on the genes involved in fat metabolism. By studying this process in mice and in laboratory-grown mouse cells, scientists discovered that PGC-1alpha activation requires not just AMPK, but the coordinated effort of AMPK and SIRT1. When cells were treated to reduce their levels of SIRT1, AMPK could not activate PGC-1alpha, and genes involved in fat metabolism were not turned on. Previous studies have shown that SIRT1 can turn on PGC-1alpha by removing specific chemical modifications that normally inhibit its function. The researchers in this study found that this "modifying" function of SIRT1 was activated indirectly by AMPK, where AMPK raises the amount of a small cellular signaling molecule that is required for SIRT1 function. Although AMPK and SIRT1 have both previously been implicated in PGC-1alpha activation, this study provided the first evidence that they act together in a coordinated fashion to regulate energy expenditure.

In the second study, researchers discovered that SIRT1 also plays a key role in glucose (sugar) metabolism by regulating the response to insulin in adipose (fat) tissue. When they compared mice with diet-induced obesity to "normal" mice, the researchers observed that the levels of SIRT1 in adipose tissue were dramatically decreased in the obese mice. Using laboratory-grown adipose cells, the scientists then showed that a decrease in SIRT1 protein levels correlated with impaired insulin signaling and the decreased ability of adipose cells to take up glucose. In addition to promoting insulin resistance, SIRT1 deficiency also appears to result in a state of chronic inflammation: The scientists found that SIRT1 protein acts in an anti-inflammatory fashion by removing chemical modifications from, and thereby inactivating, NF-kappaB, a key protein that turns on an inflammatory response. When the researchers suppressed the inflammatory response, either by reducing NF-kappaB protein levels or by using pharmacologic agents that activate SIRT1 (and, thus, inactivate NF-kappaB), they found that adipose cells have improved insulin signaling and improved insulin-stimulated glucose uptake. These results put SIRT1 in the center of a pathway connecting insulin resistance and inflammation. Based on their anti-inflammatory effect, activators of SIRT1 may turn out to be a useful therapeutic intervention for improving insulin sensitivity as a means of treating type 2 diabetes.

Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, and Auwerx J: AMPK regulates energy expenditure by modulating NAD + metabolism and SIRT1 activity. Nature 458: 1056-1060, 2009.

Yoshizaki T, Milne JC, Imamura T, Schenk S, Sonoda N, Babendure JL, Lu JC, Smith JJ, Jirousek MR, and Olefsky JM: SIRT1 exerts anti-inflammatory effects and improves insulin sensitivity in adipocytes. Mol Cell Biol 29: 1363-1374, 2009.

The Right To Assemble—Aggregates of Hormones in Health and Disease: Scientists discovered a normal biological function for a type of protein aggregate generally associated with disease. Type 2 diabetes, like Alzheimer's disease and some other neurodegenerative diseases, is associated with the appearance of "amyloids" in a patient's damaged tissues—the pancreatic islet cells in diabetes and the brain in Alzheimer's disease. Amyloids are defined by their structure—characteristic filamentous

aggregates—rather than by the specific proteins that form them. It is unknown whether amyloids are a cause or a consequence of the diseases associated with them.

In this study, the scientists found that certain hormones can be stored as amyloids in a highly organized and concentrated form. When the scientists exposed these hormone amyloids to conditions similar to those that exist during hormone secretion, they disassembled, releasing the hormones as would be required if the amyloids serve as storage depots in advance of secretion. Interestingly, the scientists showed that the effects can be toxic if the amyloid packages are opened at the wrong time or place. They hypothesized that conditions like diet, stress, or age could alter the physiological balance that regulates the release of hormone amyloids and lead the amyloids to collect and aggregate, as seen in disease. In the pancreas, this build-up of amyloids might induce the insulin-producing beta cells of the pancreas to die, leading to the dramatic loss of beta cells seen in type 2 diabetes. Additional research is necessary to test these hypotheses and to better understand the role of amyloids in health and disease, but this exciting result provides an important insight in basic hormone biology.

Maji SK, Perrin MH, Sawaya MR, Jessberger S, Vadodaria K, Rissman RA, Singru PS, Nilsson PR, Simon R, Schubert D, Eisenberg D, Rivier J, Sawchenko P, Vale W, and Riek R: Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. Science 325: 328-332, 2009.

Neuro-Protective Peptide May Also Regulate Insulin Sensitivity: A small protein that can protect brain cells from death in Alzheimer's disease may also open up new avenues to improving insulin action in people with type 2 diabetes. The protein, called humanin, promotes brain cell survival in the face of Alzheimer's disease and other forms of brain injury. Because there is increasing evidence for an association between insulin resistance and Alzheimer's disease, scientists hypothesized that humanin might also positively affect insulin sensitivity.

Using a technique called insulin clamping, which allows scientists to observe changes in insulin sensitivity under different experimental conditions (such as normal or high levels of insulin), the researchers tested whether humanin could affect insulin sensitivity in rodent models. They found that rats that were treated with humanin in the hypothalamus—a part of the brain that helps govern the rest of the body's response to insulin—showed increased insulin sensitivity over rats that received a control treatment. This positive effect was seen even in rats that had experimentally induced high levels of insulin (hyperinsulinemia), which in humans is a symptom of insulin resistance. In the hyperinsulinemic rats, infusing humanin into the brain increased insulin sensitivity in both major target tissues of insulin action, liver and muscle. When delivered intravenously instead of into the brain, "normal" humanin did not increase insulin sensitivity in the rats, but a specially modified, more potent form of the protein did—demonstrating that humanin's effect on insulin sensitivity may be achieved without direct administration into the brain. In diabetic rats, a single intravenous injection of the more potent form of humanin was able to reduce the animals' blood glucose levels for several hours.

Insulin resistance is a feature of many diseases and disorders, including type 2 diabetes, pre-diabetes, obesity, and cardiovascular disease. This study adds to the evidence for its association with Alzheimer's disease and other brain diseases as well. While these experiments were conducted in rodents, and more remains to be learned about the role of humanin in regulating insulin action, the study findings may point toward new approaches to treating type 2 diabetes.

Muzumdar RH, Huffman DM, Atzmon G, Buettner C, Cobb LJ, Fishman S, Budagov T, Cui L, Einstein FH, Poduval A, Hwang D, Barzilai N, and Cohen P: Humanin: a novel central regulator of peripheral insulin action. PLoS One 4: e6334, 2009.

Protein Factor Allows Fructose To Fuel Metabolic Diseases: New research has identified a molecular connection between consumption of the dietary sugar, fructose, and metabolic problems, including type 2 diabetes. Increased consumption of fructose, commonly used as a sweetener in sodas and processed foods, has roughly paralleled the dramatic increases in overweight and obesity observed since the 1970s. Although it is thought that a complex interplay of many factors has

driven the obesity epidemic in the U.S., the widespread use of fructose has been proposed as a potential contributing factor. Fructose consumption has been linked to metabolic abnormalities such as nonalcoholic fatty liver disease and type 2 diabetes. Further, fructose has been shown to be a more potent trigger of fat production in the body than other sugars, although the reasons for this have been unclear.

New research has implicated the protein PGC-1beta as playing a key role in mediating the metabolic effects of fructose. When rats are fed a diet rich in fructose, they develop symptoms associated with the metabolic syndrome and type 2 diabetes in humans, including weight gain, an unhealthy blood lipid profile, and high blood glucose and insulin levels. Researchers experimentally diminished PGC-1beta in rats fed a high-fructose diet, and found that this protected the rats from fructose-induced weight gain, as well as other metabolic abnormalities linked to fructose, such as insulin resistance and excessive production of glucose by the liver. Diminishing PGC-1beta conferred a more modest benefit in rats with insulin resistance due to a high-fat diet. In contrast, reducing the amount of PGC-1beta in rats fed a standard diet actually increased their liver glucose production. These results suggest that PGC-1beta promotes the metabolic problems associated with a high-fructose diet. The data also support the idea that reducing consumption of fructose may help some people avoid overweight, obesity, and some of the diseases associated with them, and suggest that a therapeutic approach targeting PGC-1beta may be beneficial for some people with metabolic syndrome or type 2 diabetes.

Nagai Y, Yonemitsu S, Erion DM, Iwasaki T, Stark R, Weismann D, Dong J, Zhang D, Jurczak MJ, Löffler MG, Cresswell J, Yu XX, Murray SF, Bhanot S, Monia BP, Bogan JS, Samuel V, and Shulman GI: The role of peroxisome proliferator-activated receptor gamma coactivator-1 beta in the pathogenesis of fructose-induced insulin resistance. Cell Metab 9: 252-264, 2009.

Keeping an Even Energy Keel from Meal to Meal: Research in mice is providing new insights into the way the mammals regulate their energy supply. The body needs energy at all times to keep cells and organs functioning. But the supply of energy from food is not constant. Eating typically provides more fuel than is necessary at precisely that moment, so the excess must be appropriately stored. In contrast, periods of fasting and starvation require liberation of a sufficient supply of stored energy to keep the organism going until the next meal—which may be hours, days, or weeks away. New discoveries are identifying the molecular triggers that control mammalian responses to a varying energy supply.

One study showed that insulin, the hormone that instructs cells to take up glucose following feeding, triggers a protein called DNA-PK to modify another protein called USF-1, which then activates genes involved in storing energy as fat. This is surprising, because DNA-PK was known for playing a key role in repairing genetic damage, but had not previously been understood to play a part in metabolism.

When a mammal is eating regularly, a ready energy supply in the liver keeps its body going between meals. When the length of time between feedings is extended long enough to deplete that ready supply, however, the body enters starvation mode and must avail itself of other energy depots. A second study identified molecular factors in this fasting-to-starvation transition. Researchers found that a hormone called FGF21 induces the liver to make the protein PGC-1 alpha, which then turns on genes controlling processes like the burning of fat for fuel and the production of glucose from other energy stores.

These results improve understanding of metabolic regulation in healthy mammals—regulation that may go awry in disease. Such fundamental knowledge may also lead to improved treatment for metabolic diseases, including diabetes and obesity.

Wong RH, Chang I, Hudak CS, Hyun S, Kwan HY, and Sul HS: A role of DNA-PK for the metabolic gene regulation in response to insulin. Cell 136: 1056-1072, 2009.

Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA, and Burgess SC: FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. Proc Natl Acad Sci USA 106: 10853-10858, 2009.

To Understand Bone Formation, Just Follow Your Gut: Scientists made a surprising discovery that bone formation is regulated by levels of gut-derived serotonin, and a gene called *Lrp5* controls bone formation by inhibiting serotonin production. Bone is living tissue that constantly rebuilds as old bone tissue is broken down and new bone is formed. The *Lrp5* gene had previously been found to be important in bone formation. Mice lacking *Lrp5* have low bone mass due to a decrease in bone formation. In people, mutations in this gene are associated with bone diseases, including a form of osteoporosis. However, it was unknown how *Lrp5* regulated bone formation, whether it was acting directly on the bone, and what other cellular factors were involved.

To understand how *Lrp5* regulates bone formation, scientists first sought to identify factors controlled by *Lrp5* by determining whether any genes were turned on or off differently in bones of mice lacking *Lrp5* as compared to normal mice. In mice lacking *Lrp5*, they found that the gene turned on to the greatest extent is involved in serotonin synthesis, and the activity of this gene was also increased dramatically in gut cells. The animals also had abnormally high levels of serotonin. Only about 5 percent of the body's serotonin is produced in the brain, where it modulates mood, appetite, sleep, and other processes. The other 95 percent is made in the duodenum of the gastrointestinal tract, but the function of this gut-derived serotonin has been a matter of scientific debate.

With these clues about the importance of gut-derived serotonin, the scientists performed a series of experiments in mice to examine serotonin's role in bone formation. In one experiment, they found that administration of a chemical inhibitor of serotonin synthesis normalized bone formation in mice lacking *Lrp5*. In another experiment, they discovered that genetically turning off serotonin production in the gut protected against bone loss in a mouse model of menopause (a time period when women are at greater risk for loss of bone density). Overall, the research showed that increasing serotonin levels slowed the formation of new bone, while inhibiting serotonin production promoted it. In addition, the research demonstrated that Lrp5 was not acting directly on bone, but rather in the gut to regulate production of serotonin, which in turn travels through the body to inhibit bone formation. Studies in a small number of people with bone diseases associated with mutations in *LRP5* showed that the patients have abnormal serotonin levels. These preliminary observations suggest that serotonin may also be important in controlling bone formation in people. Most drugs for osteoporosis that are approved for use in people prevent the breakdown of bone, but do not promote the generation of new bone. The discovery that gut-derived serotonin inhibits bone formation in mice and possibly in people suggests that therapies to inhibit serotonin production in the gut, or to block its action on bone, could be a novel means by which to treat or preempt osteoporosis.

Yadav VK, Ryu JH, Suda N, Tanaka KF, Gingrich JA, Schütz G, Glorieux FH, Chiang CY, Zajac JD, Insogna KL, Mann JJ, Hen R, Ducy P, and Karsenty G: Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. Cell 135: 825-837, 2008.

CIRCADIAN RHYTHM AND METABOLISM

Circadian Rhythm and Metabolism Depend on Changes in Gene Activity Caused by Histone Modifications: A newly uncovered link between circadian rhythm and metabolism may yield therapeutic targets for metabolic diseases. In animals and humans, the circadian clock regulates many behaviors and bodily processes—including sleep/wake cycles, changes in blood pressure, and body temperature fluctuations—to harmonize these activities with daily, rhythmic changes in the environment, most notably day/night cycles. The circadian clock also has a critical relationship with metabolic pathways important to maintaining normal energy balance. Identifying the molecular signals that link circadian rhythm and metabolism could yield important insights into metabolic disorders and diseases such as insulin resistance, diabetes, and obesity and how to thwart their development. Scientists now have evidence that interactions between circadian clock genes and metabolic genes are governed by transitory changes to the proteins associated with DNA, called "histones," and have identified two key factors in these interactions. By chemically modifying histones, the cell is able to physically "open up" or "close down" access to underlying DNA and thereby dynamically regulate gene expression (whether genes are turned "on" or "off").

In a new study in mouse models, researchers used genetic techniques to disrupt the interaction between a histone-modifying protein and a partner protein that both recruit the histone-modifying protein to circadian genes and activate it. They found that the mutant mice had abnormal patterns of circadian gene expression, and their circadian behaviors were disrupted. More significantly, the mutant mice grew to be leaner than their normal littermates, more sensitive to insulin, and resistant to obesity from a high-fat diet. Molecular studies revealed that the cyclic expression of several metabolic genes had been significantly altered in the mutant mice, reinforcing that the timing of expression of metabolic genes is critical for normal energy balance. Although these studies were carried out in mice, the results suggest that altering circadian rhythm by inducing a different pattern of histone-modification at circadian clock genes can potentially have positive metabolic effects. Future studies may exploit these findings to more precisely define the changes that yield metabolic benefit and develop new approaches to help prevent metabolic diseases.

Alenghat T, Meyers K, Mullican SE, Leitner K, Adeniji-Adele A, Avila J, Bućan M, Ahima RS, Kaestner KH, and Lazar MA: Nuclear receptor corepressor and histone deacetylase 3 govern circadian metabolic physiology. Nature 456: 997-1000, 2008.

The Rhythm of Metabolism: Scientists have identified a genetic link between fasting glucose levels and melatonin, a molecule that regulates circadian rhythms. The circadian rhythm is a roughly 24-hour cycle that humans and other organisms use to anticipate changes in their external environment, such as light and dark, and thus establish sleeping and feeding cycles. Previous studies uncovered a relationship between circadian rhythms and the body's metabolism, leading scientists to hypothesize that metabolic disorders, including obesity and type 2 diabetes, might be linked to disruption of circadian rhythms.

Elevation of blood glucose levels is associated with diabetes, but even among people who do not have diabetes there is variation in fasting blood glucose levels. Researchers recently performed a genome-wide association study to scan a set of common genetic variations throughout the genome for those associated with high or low fasting glucose levels. Identification of such variations could help scientists understand how glucose levels are regulated in the body and how these levels can become unregulated in disease. Genetic data from several previous studies were combined, so that this study effectively included over 36,000 individual genomes from people of European descent. The researchers found that a variant in a gene called *melatonin receptor 1B* (*MTNR1B*) was consistently associated with elevated fasting glucose levels. In addition, this variant was associated with reduced pancreatic beta cell function. Beta cells, which synthesize and secrete insulin, are critical for normal control of glucose levels. Moreover, the variant was associated with an increased risk of development of type 2 diabetes.

The product of the *MTNR1B* gene is known to interact with melatonin. Melatonin levels are lowest during the day and peak at night, whereas insulin levels drop at night. This suggests that insulin levels may be controlled, at least in part, by an inhibitory effect of melatonin on insulin secretion. Thus, the identification of *MTNR1B* as a factor in fasting blood glucose levels helps explain the relationship between glucose regulation and circadian rhythms, and provides evidence in humans for a link between melatonin and type 2 diabetes. Interestingly, fasting blood glucose is typically measured in the morning, so that study participants do not have to miss daytime meals. This may have helped the researchers to identify the circadian link. By shedding light on the biology of glucose metabolism, this study may eventually help lead to a new therapeutic avenue to treat people with type 2 diabetes.

Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, Potter SC, Erdos MR, Sanna S, Hottenga JJ, Wheeler E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, Bonnycastle LL, Buchanan TA, Cao A, Cervino A, Coin L, Collins FS, Crisponi L, de Geus EJ, Dehghan A, Deloukas P, Doney AS, Elliott P, Freimer N, Gateva V, Herder C, Hofman A, Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, Naitza S, Orrù M, Palmer CN, Pouta A, Randall J, Rathmann W, Saramies J, Scheet P, Scott LJ, Scuteri A, Sharp S, Sijbrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuomi T, Tuomilehto J, Uitterlinden AG, Voight BF, Waterworth D, Wichmann

HE, Willemsen G, Witteman JC, Yuan X, Zhao JH, Zeggini E, Schlessinger D, Sandhu M, Boomsma DI, Uda M, Spector TD, Penninx BW, Altshuler D, Vollenweider P, Jarvelin MR, Lakatta E, Waeber G, Fox CS, Peltonen L, Groop LC, Mooser V, Cupples LA, Thorsteinsdottir U, Boehnke M, Barroso I, Van Duijn C, Dupuis J, Watanabe RM, Stefansson K, McCarthy MI, Wareham NJ, Meigs JB, and Abecasis GR: Variants in MTNR1B *influence fasting glucose levels. Nat Genet 41: 77-81, 2009.*

NEW SCREENING TEST FOR INBORN ERRORS OF METABOLISM

Improved Test Offers Hope for Children Born with Mucopolysaccharidosis I: A new diagnostic test may help doctors identify babies born with the lysosomal storage disorder mucopolysaccharidosis I (MPS I) early enough to provide optimal therapy. The body's cells recycle many of the substances they no longer need by digesting them with enzymes inside cellular compartments called lysosomes. If these enzymes are missing or defective due to genetic mutations, toxic waste products are not properly degraded. Instead, they build up in the lysosomes and lead to severe organ damage. Diseases caused by these enzyme deficiencies are referred to collectively as lysosomal storage disorders. Symptoms vary, and are often not apparent at birth. However, as the undigested materials accumulate, they can cause serious problems such as weakness, severe pain, brittle bones, mental retardation, corneal clouding, organ failure, and death. In recent years, scientists discovered that many of these symptoms can be alleviated by administering the missing enzyme directly to affected patients. However, in order for this type of therapy to confer maximal benefit, it is important to start treatment early on in a patient's life. The new test can allow clinicians to diagnose the disorder before symptoms develop by analyzing a spot of dried blood for the activity of the enzyme missing in MPS I. The researchers who developed the test had sought to improve on a previous diagnostic assay they had developed, and note that features of the new test make it easier and more practical to use. If further evaluation confirms the utility of this new test for newborn screening, it may enable enzyme replacement therapy or bone marrow transplant to take place early enough to avert many of the most serious symptoms of the disease.

Blanchard S, Sadilek M, Scott CR, Turecek F, and Gelb MH: Tandem mass spectrometry for the direct assay of lysosomal enzymes in dried blood spots: Application to screening newborns for mucopolysaccharidosis I. Clin Chem 54: 2067-2070, 2008.

CYSTIC FIBROSIS RESEARCH

Genetic Risk Factor Identified for Liver Disease Development in Cystic Fibrosis: An international scientific collaboration has resulted in the discovery of the first genetic risk factor for severe liver disease development in some people with cystic fibrosis (CF). CF is an inherited disease that affects mainly the lungs, pancreas, and sweat glands. However, many individuals with CF also develop abnormal liver function and fibrosis, with some progressing to severe liver disease. The major genetic defects responsible for CF are mutations in the gene called *CFTR*, which result in mucus accumulation in airways and abnormal function of other organs. However, the process by which liver disease develops in individuals with CF is unclear, and patients with the mutated *CFTR* gene exhibit a wide range of disease severity. Based on these observations, additional genetic factors likely play a role in determining susceptibility to liver disease development in patients with CF. Currently, no diagnostic test exists to identify which individuals with CF are at high risk of developing severe liver disease.

Researchers, at sites around the globe, worked together to identify genetic risk factors associated with severe liver disease development in CF. They increased their chances of finding these genetic factors by performing two sequential studies of patients with CF and healthy controls: an initial study examining five genes suspected of contributing to CF liver disease, followed by a second study to confirm these genetic associations. In the initial study, two genetic variants, one in the *SERPINA1* gene, referred to as the *SERPINA1 Z* allele, and another in the *TGFB1* gene, were associated with CF liver disease; however, the second study confirmed only *SERPINA1 Z* allele as a risk factor. When the data from both studies were combined for greater statistical power, the *SERPINA1 Z* allele was shown to have a strong association with CF liver disease. The *SERPINA1 Z* allele is known to cause proteins to fold incorrectly and accumulate in liver cells; however the mechanism by which it contributes to CF liver disease requires further study.

This research identifies the *SERPINA1 Z* allele as the first genetic factor that increases the risk of developing severe liver disease in individuals with CF. This discovery allows for the possibility of future infant screening programs that follow up on diagnosis of CF by testing for their genetic susceptibility to develop severe liver disease later in life, so that appropriate preemptive action can be taken.

Bartlett JR, Friedman KJ, Ling SC, Pace RG, Bell SC, Bourke B, Castaldo G, Castellani C, Cipolli M, Colombo C, Colombo JL, Debray D, Fernandez A, Lacaille F, Macek M Jr, Rowland M, Salvatore F, Taylor CJ, Wainwright C, Wilschanski M, Zemková D, Hannah WB, Phillips MJ, Corey M, Zielenski J, Dorfman R, Wang Y, Zou F, Silverman LM, Drumm ML, Wright FA, Lange EM, Durie PR, and Knowles MR, for the Gene Modifier Study Group: Genetic modifiers of liver disease in cystic fibrosis. JAMA 302: 1076-1083, 2009.

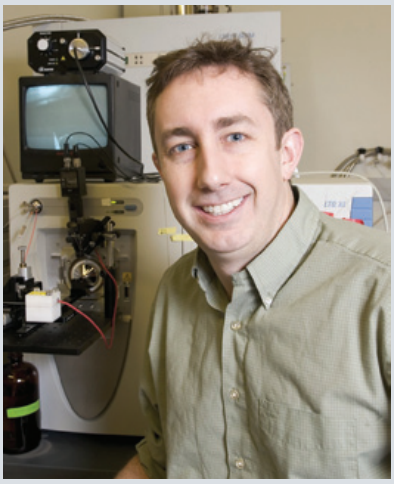
Improved Treatment for Diabetes Related to Cystic Fibrosis: A new study has revealed that a diabetes treatment can benefit many patients with an increasingly common complication of cystic fibrosis. CF, a genetic disorder that leads to chronic lung infections, once led inevitably to childhood death from scarring of the lungs. New treatments are helping people with CF live much longer—often into their 30s and 40s. However, as they age, an increasing number of people with CF are developing CF-related diabetes (CFRD), which has been associated with reduced survival. CF severely damages the pancreas, affecting first its vital role in producing digestive enzymes needed for food absorption from the intestine, and later its production of insulin needed to transport glucose fuel into cells. Replacement of lost digestive enzymes improves growth and nutrition, but sufficient insulin is also needed to maintain body weight and muscle mass. Still, because patients with CFRD may not be at risk for many of the serious complications associated with other forms of diabetes, the benefit of adding insulin therapy to the already burdensome CF treatment regimen has been uncertain. A recent clinical trial has now shown that aggressive insulin therapy, begun earlier in the course of their diabetes than previously recommended, can help many people with CFRD maintain their body weight and potentially avoid the excess mortality associated with CFRD.

Moran A, Pekow P, Grover P, Zorn M, Slovis B, Pilewski J, Tullis E, Liou TG, Allen H, and the Cystic Fibrosis Related Diabetes Therapy Study Group: Insulin therapy to improve BMI in cystic fibrosis-related diabetes without fasting hyperglycemia: results of the cystic fibrosis related diabetes therapy trial. Diabetes Care 32: 1783-1788, 2009.

Dr. Michael J. MacCoss and Dr. Kristin V. Tarbell: NIDDK-Supported Scientists Receive Presidential Award

Two scientists supported by NIDDK have received the Presidential Early Career Award for Scientists and Engineers (PECASE). PECASE is awarded annually to scientists and engineers who, while early in their research careers, have demonstrated the pursuit of innovative research and outstanding scientific leadership. Among the recipients in 2007 was Michael J. MacCoss, Ph.D., an NIDDK extramural grantee, and in 2008, Kristin V. Tarbell, Ph.D., a scientist in NIDDK's Division of Intramural Research. In addition to the NIDDK-supported recipients, in both years, 11 other scientists supported by the NIH received the award for their scientific achievements; NIH has now funded 153 PECASE recipients since the award's inception in 1996. PECASE is the most prestigious award given in the U.S. to scientists at the outset of their independent research careers.

Developing Cutting-Edge Technologies To Study Health and Disease



Dr. Michael J. MacCoss

Dr. MacCoss, an Assistant Professor in the Department of Genome Sciences at the University of Washington in Seattle, received a 2007 PECASE award for his innovative work in the emerging field of "proteomics"—the large-scale, "big-picture" view of all of the proteins that make up a cell. Of interest to Dr. MacCoss, however, is not so much what proteins are in a cell, but rather the dynamics of how quickly proteins are made and how quickly they are destroyed. The balance of making and destroying proteins is an essential way to control how much time a protein can spend carrying out its specific cellular function, an important aspect of normal health and disease. To facilitate the study of "quantitative proteomics," as it is known, Dr. MacCoss' research group is developing innovative technologies that will allow scientists to map out the dynamic life cycle, or turnover, of proteins within a cell. His team is applying these tools to model organisms to study how insulin—a hormone that regulates uptake of glucose by a cell—affects the turnover of proteins involved in cellular metabolism. As insulin and a cell's ability to respond to insulin are intimately linked to type 2 diabetes, Dr. MacCoss' studies may provide new fundamental insight into how protein turnover regulates the cellular response to insulin and alters the response in type 2 diabetes.

Understanding Immune Tolerance and Type 1 Diabetes

Dr. Kristin V. Tarbell

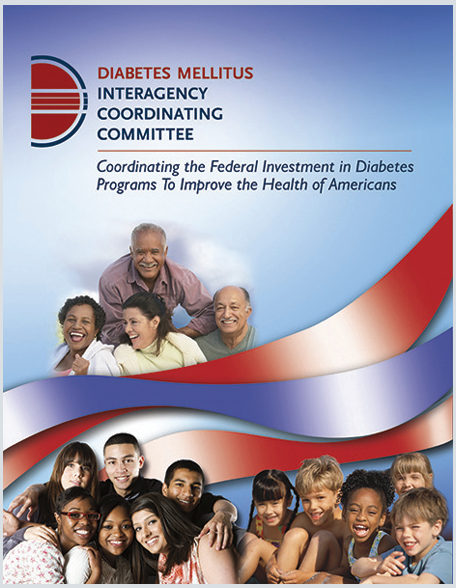
Dr. Tarbell, a tenure-track investigator in the Diabetes Branch of NIDDK's Division of Intramural Research, received a 2008 PECASE for her seminal research studying immune tolerance and autoimmunity. The human immune system protects the body from foreign, potentially harmful molecules and organisms. Since the body does not want to attack itself, the immune system

has a set of suppressive features, known as immune tolerance, that prevent its inappropriate activation. When these mechanisms are compromised, however, the aberrant attack of "self" often results in autoimmune diseases such as type 1 diabetes. While a Research Associate at Rockefeller University, where she received an NIDDK Mentored Research Scientist Development Award, Dr. Tarbell made important discoveries regarding how two types of immune cells—dendritic cells (DCs) and regulatory T cells (Tregs)—interact to suppress the autoimmune response that causes type 1 diabetes. Importantly, Dr. Tarbell and her colleagues showed that Tregs, when activated by DCs, can be used to block the development of diabetes or reverse diabetes in mice. Since joining NIDDK, Dr. Tarbell and her research group have continued to explore how both DCs and Tregs can be used to suppress the autoimmune response that causes type 1 diabetes. Future advances from Dr. Tarbell's research should provide fundamental insight on how autoimmunity develops, which may translate into new therapeutic approaches for reversing type 1 diabetes in patients.

The PECASE awards support the continued professional development of awardees, promote careers and foster innovation in science and technology, and recognize the scientific missions of participating agencies. A list of NIH scientists who have received this prestigious award is available at *www.grants.nih.gov/grants/policy/pecase.htm*

Diabetes Mellitus Interagency Coordinating Committee: Coordinating the Federal Investment in Diabetes Programs To Improve the Health of Americans

Diabetes places huge personal and economic burdens on Americans. However, even as rates of diabetes are rising, people with diabetes are living longer and healthier lives. Advances in medicine, public health, and health care have led to significant progress. New research discoveries and translation efforts will yield further improvements in the prevention, diagnosis, and treatment of diabetes. Building on the accomplishments and successes of Federal programs in improving public health with regard to diabetes, the government agencies responsible for leading the Federal investment in diabetes are working together to improve the health of Americans.



Congress created the statutory Diabetes Mellitus Interagency Coordinating Committee (DMICC) in 1974 with the charge to coordinate diabetes research activities and health programs of the National Institutes of Health and other Federal agencies, and to provide for the communication and exchange of information necessary for coordination. Chaired by the NIDDK, the DMICC has 35 members representing diverse agencies within the U.S. Department of Health and Human Services (DHHS), the U.S. Department of Defense, the U.S. Department of Agriculture, and the Veterans Health Administration. These member organizations have important, unique, and complementary roles in the Federal diabetes effort to improve public health.

Individual Federal agencies have made significant, measurable strides in combating diabetes. However, many successful efforts have required a combination of expertise and resources not found in a single organization. Interagency coordination through the DMICC is thus essential to avoid unnecessary duplication of diabetes activities, to maximize the value of available resources, and to ensure the optimal use of federal funds to combat and alleviate the public health burden of diabetes. The DMICC coordinates federal diabetes activities and works to share information, foster joint efforts, and identify opportunities for agency collaboration.

Since its inception, the DMICC has facilitated successful, collaborative diabetes activities among its member organizations. For example, the National Diabetes Education Program, the leading Federal Government public education program that promotes diabetes prevention and control, is jointly overseen by the NIDDK and Centers for Disease Control and Prevention. Another example, the landmark Diabetes Prevention Program clinical trial, which dramatically showed that type 2 diabetes can be prevented or delayed, involved many DMICC member organizations. *The National Diabetes Fact Sheet*, a product of collaborations among DMICC member organizations, summarizes the latest estimates of Americans with pre-diabetes and with diagnosed and undiagnosed diabetes, helping those at Federal, State, and local levels understand the health and economic

burden of diabetes. Further highlights of major areas of successful collaboration include coordination of the trans-DHHS *Special Statutory Funding Program for Type 1 Diabetes Research* and strategic planning for diabetes research programs.

In response to the immense and growing public health burden of diabetes, the DMICC has been expanding and enhancing its efforts. The DMICC is uniquely poised to leverage Federal resources, reduce redundancy of effort, and increase public awareness of Federal diabetes research, programs, and health information to combat the diabetes epidemic.

The DMICC recently developed a booklet to increase awareness of the Committee and the many diabetes activities coordinated by its member organizations. Entitled "DMICC: Coordinating the Federal Investment in Diabetes Programs To Improve the Health of Americans," the new booklet includes information about the Committee, its member organizations, the coordination of Federal diabetes efforts, and the activities and successes of the Committee. The Committee's booklet is available in electronic form through its Web site: *www.diabetescommitee.gov* Hard copies of the publication are available through the National Diabetes Information Clearinghouse at: *http://catalog.niddk.nih.gov*

The Improved Outlook for People with Type 1 Diabetes

In the 1950s, about one in five people died within 20 years after a diagnosis of type 1 diabetes. About one in four people developed kidney failure within 25 years of diagnosis. About 90 percent of people with type 1 diabetes developed diabetic retinopathy within 25 years of diagnosis. People monitored their blood glucose levels with urine tests, which recognized high, but not dangerously low, glucose levels and reflected past, not current, glucose levels.

Today, the outlook for people with type 1 diabetes is greatly improved due to landmark studies that demonstrated the importance of early intensive blood glucose control and due to improvements in technology. The NIDDK's landmark Diabetes Control and Complications Trial (DCCT) and its follow-up study, the Epidemiology of Diabetes Intervention and Complications (EDIC), recently demonstrated that near-normal control of glucose beginning as soon as possible after diagnosis can greatly improve the long-term prognosis of type 1 diabetes. The study also found that the prognosis for people with longstanding type 1 diabetes has greatly improved in the past 20 years due to a better understanding of the importance of intensive glucose control, as well as advances in insulin formulations, insulin delivery, glucose monitoring, and the treatment of cardiovascular risk factors.

The DCCT, conducted from 1983 to 1989, compared intensive management of blood glucose to conventional control in 1,441 people 13 to 39 years of age with recently diagnosed type 1 diabetes. At the time, conventional treatment consisted of one or two insulin injections a day with daily urine or blood glucose testing. Participants randomly assigned to intensive treatment were asked to keep glucose levels as close to normal as possible. That meant trying to keep hemoglobin A1c (HbA1c) readings at 6 percent or less with at least three insulin injections a day or an insulin pump, guided by frequent self-monitoring of blood glucose. (HbA1c reflects average blood glucose levels over the previous 2 to 3 months.)

The DCCT found that intensive glucose control was superior to conventional control in delaying or preventing the complications of type 1 diabetes. EDIC continues to follow DCCT participants to determine the long-term effects of prior intensive versus conventional blood glucose control. In the most recent study, the researchers compared overall rates of eye, kidney, and cardiovascular complications in three groups of people diagnosed with type 1 diabetes an average of 30 years earlier.[1] Two groups consisted of DCCT/EDIC participants—those randomly assigned to intensive glucose control or to conventional control. The third group was a subset of patients in the NIDDK-supported Pittsburgh Epidemiology of Diabetes Complications (EDC) study. The EDC is a population-based study that has been following residents of Allegheny County, Pennsylvania, who were diagnosed with type 1 diabetes from 1950 to 1980.

After 30 years of diabetes, DCCT participants randomly assigned to intensive glucose control had about half the rate of eye damage compared to those assigned to conventional glucose control (21 percent vs. 50 percent). They also had lower rates of kidney damage (9 percent vs. 25 percent) and cardiovascular events (9 percent vs. 14 percent) compared to those receiving conventional glucose control. The intensively treated DCCT group also had lower complication rates than EDC participants, whose rates were similar to the DCCT's conventional group. These observations suggest that implementing intensive glucose control as early in the course of diabetes as possible could help people avoid the life-threatening complications that were much more common in the past.

Major improvements in glucose monitoring and insulin delivery introduced in the past decade are now helping patients control their blood glucose more precisely and conveniently and reduce the risk of hypoglycemia. For example, several continuous glucose monitoring devices approved by the U.S. Food and Drug Administration give both trend and real-time information on glucose levels. Insulin pump technology is also improving, and researchers have begun testing a system that combines

both technologies in patients with newly diagnosed type 1 diabetes. With early intensive therapy to control blood glucose levels and improvements in technology, the outlook for people diagnosed with type 1 diabetes is better than ever.

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[1] *Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group, Nathan DM, Zinman B, Cleary PA, Backlund JY, Genuth S, Miller R, and Orchard TJ: Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complications experience (1983-2005). Arch Intern Med 169: 1307 -1316, 2009.*

STORY OF DISCOVERY

Research Provides the Power To Prevent Type 2 Diabetes

In the early 1990s, public health experts recognized a developing type 2 diabetes crisis. Rising rates of obesity and an aging populace were driving worldwide prevalence of the disease higher—fast. In many parts of the world that had previously recorded low rates of diabetes, alteration of traditional lifestyles was leading to a diabetes explosion. As an extreme example, rates of type 2 diabetes in the Pima Indians of Arizona, aged 30 to 64—among whom the disease was once all but unknown—exceeded 50 percent in 1994.[1] Many racial and ethnic groups, including African Americans, Alaska Natives, American Indians, Asian Americans, Hispanics/Latinos, and Pacific Islanders, are disproportionately impacted by type 2 diabetes. Rates of type 2 diabetes are climbing in much of the world, but are particularly high in the U.S., where about 24 million people now have diabetes.

What was lacking in the early 1990s was any proven way to do something about the problem; although type 2 diabetes was linked to the obesity epidemic, no one knew whether losing weight would prevent diabetes, or how much weight needed to be lost. In addition, while there were several drugs available to treat diabetes once it developed, no one knew whether any of these could also prevent the disease. Scientists did know how to identify people at elevated risk of type 2 diabetes. Some people have "impaired glucose tolerance" (IGT)—their bodies have become somewhat resistant to the effects of insulin, so they are unable to produce enough of the hormone to keep glucose at a normal level in the blood. This condition places people at substantially higher risk of developing diabetes in the future than people whose blood glucose stays within the normal, healthy range. For this reason, IGT is sometimes called "pre-diabetes." People who have this condition and who are also overweight are a group that scientists hope to help with a diabetes prevention strategy.

The enormous costs associated with type 2 diabetes, including premature death, blindness, kidney failure, amputation, and heart disease, as well as its economic burden, are compounded by the progressive difficulty of treating type 2 diabetes with longer duration of disease. These factors have made type 2 diabetes a critical target for prevention. The dramatic rise in diabetes among the Pima and other American Indian groups strongly suggested that a change in lifestyle had precipitated the problem, and if so, a change in lifestyle might also help to alleviate it. Therefore NIDDK researchers and grantees proposed to test whether changes in diet and physical activity designed to yield modest weight loss might reduce the risk of type 2 diabetes in those at risk. That idea came to be the Diabetes Prevention Program (DPP) clinical trial.

The Diabetes Prevention Program

In the DPP, adult participants from 27 clinical centers around the U.S. were randomly divided into different treatment groups. The first group, called the lifestyle intervention group, received intensive training in diet, physical activity, and behavior modification. By eating less fat and fewer calories and doing moderate exercise, such as brisk walking, for a total of 150 minutes a week, they aimed to lose 7 percent of their body weight and maintain that loss. This intervention was based on extensive behavioral research that suggested it would be a sustainable approach to modest weight loss for a high proportion of participants. The second group took the generic diabetes drug metformin twice a day. The third, a control group, received placebo pills instead of metformin. The metformin and placebo groups

also received information about diet and exercise, but no intensive behavior change counseling. A fourth group was treated with the drug troglitazone, but this part of the study was discontinued after researchers discovered that troglitazone can cause serious liver damage. The participants in this last group were followed but not included as one of the intervention groups. All 3,234 study participants were overweight or obese and had pre-diabetes. Forty-five percent of the participants belonged to racial and ethnic groups at increased risk for developing diabetes, including the Pima Indians of the Gila River Reservation in Arizona, and three additional American Indian tribes.

The study was a tremendous success. The lifestyle intervention reduced participants' risk of developing diabetes by 58 percent. Lifestyle changes worked particularly well for participants age 60 and older, reducing their risk by 71 percent, and were equally effective for all participating ethnic groups and for both men and women. Participants taking metformin lowered their risk of developing diabetes by 31 percent. Metformin was effective for both men and women, but was most effective in people 25 to 44 years old and in those with a body mass index of 35 or higher, meaning they were at least 60 pounds overweight.

Researchers announced the initial findings of the DPP in 2001, a year earlier than scheduled, because the results were so striking. In 2002, the National Diabetes Education Program (NDEP), jointly sponsored by the NIDDK and the Centers for Disease Control and Prevention, launched a comprehensive prevention initiative, *Small Steps. Big Rewards. Prevent Type 2 Diabetes*, to translate the results of the DPP Study into public health practices (*http://ndep.nih.gov/campaigns/SmallSteps/SmallSteps_index.htm*). The campaign delivers practical, real-world tools to help people take the small steps needed to achieve the big reward of preventing or delaying type 2 diabetes.

The DPP Outcomes Study

The success of the DPP notwithstanding, DPP researchers could not say how long the benefit would endure, since the results were based on just 3 years of data. Because the benefit of the DPP lifestyle intervention was indisputable, all three groups were offered a group-based lifestyle intervention. The Diabetes Prevention Program Outcomes Study (DPPOS) began, with most of the DPP volunteers taking part. Metformin treatment continued in the metformin group, and the lifestyle intervention group was offered additional lifestyle support. By following the participants for a total of 10 years after enrollment in the DPP, researchers have concluded that the lifestyle intervention reduced the rate of developing type 2 diabetes by 34 percent compared with placebo. Study participants in the lifestyle interventions group also had fewer cardiovascular risk factors, including lower blood pressure and triglyceride levels, despite taking fewer drugs to control their heart disease risk. Treatment with metformin reduced the rate of developing diabetes by 18 percent compared with placebo over the 10 year period.[2]

Building on the Results of the DPP

Today, NIDDK is supporting translational research to find better methods for identifying people with pre-diabetes and to develop cost-effective ways of implementing the DPP lifestyle change in communities. One innovative study is working with YMCAs to deliver a DPP-based lifestyle intervention. Other research efforts, such as the Action for Health in Diabetes (Look AHEAD) clinical trial, which seeks to reduce complications in those who have the disease, has built on the success of the DPP lifestyle intervention, and achieved even greater weight loss by modifying the lifestyle intervention. Through these

and other efforts, the signal scientific achievements of the DPP continue to drive new health discoveries, while transforming the effort to prevent type 2 diabetes and its devastating complications.

[1] *Prevention of diabetes mellitus: Report of a WHO study group (ISBN: 92 4 120844 9).*

[2] *Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ, Brenneman AT, Brown-Friday JO, Goldberg R, Venditti E, and Nathan DM, for the Diabetes Prevention Program Research Group: 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. Lancet 374: 1677-1686, 2009.*

Resistin: Looking Forward and Back

Dr. Mitchell Lazar

Dr. Mitchell Lazar is the Sylvan H. Eisman Professor of Medicine and Genetics at the University of Pennsylvania School of Medicine. He is also the Chief of the University's Division of Endocrinology, Diabetes, and Metabolism, and is the Director of the Institute of Diabetes, Obesity, and Metabolism. He received his M.D. degree and a Ph.D. in Neuroscience from Stanford University's School of Medicine. Dr. Lazar's research has been supported by NIDDK since 1991, including two MERIT awards. In addition to serving on the NIDDK's Advisory Council, Dr. Lazar has been elected to the American Society of Clinical Investigation, the Association of American Physicians, the Institute of Medicine of the National Academy of Sciences, as well as the American Academy of Arts and Sciences. Dr. Lazar is the 2009 recipient of the American Society for Clinical Investigation's Stanley J. Korsmeyer Award, in recognition of his outstanding contributions to our understanding of the regulation of metabolism.

The following are highlights from the scientific presentation that Dr. Lazar gave to the NIDDK's Advisory Council in May 2009 on new understanding of the role of the hormone resistin, which was discovered in his laboratory.

The current epidemics of obesity and type 2 diabetes have occurred during a time of tremendous change in our lifestyles, particularly in regard to diet and physical activity. How these environmental changes have influenced these epidemics is an important research question. Obesity is a risk factor for the development of type 2 diabetes, but an understanding of how these two states are related at a molecular level has proved difficult to unravel. Type 2 diabetes is characterized by a change in the ability of tissues to respond to insulin, a hormone that regulates the body's use and storage of energy sources (calories from food). In this state—known as insulin resistance—insulin is less able to promote the uptake of glucose in muscle and fat, and to inhibit the production of glucose by the liver. Once tissues start to become insulin resistant, the pancreas, the organ that produces insulin, tries to compensate by secreting more insulin, but the body eventually can become even more resistant to insulin. Obesity increases risk for insulin resistance, which can lead to type 2 diabetes.

One of the keys to elucidating the relationship between obesity and insulin resistance is to understand how increased energy storage in fat cells, referred to as "adipocytes," promotes resistance to insulin in muscle and liver. In the presence of excess calories, insulin works to promote storage of the calories as fat in fat tissue and to induce transport of glucose into fat and muscle tissues. Scientists previously thought that adipose (fat) tissue was simply a storehouse, a place to put the excess. Research over the past 2 decades has revealed that adipocytes secrete hormones that affect other tissues and organs in the body. Many of these secreted proteins are involved in metabolic processes, including glucose regulation. Could they also be involved in insulin resistance? By looking at a class of type 2 diabetes drugs called thiazolidinediones that improve insulin sensitivity, scientists discovered that the insulin-sensitizing drugs worked through a protein found in adipocytes known as PPAR-gamma. Since PPAR-gamma is in fat cells, while insulin resistance occurs primarily in liver and muscle cells, the scientists thought that there must be a factor regulated by PPAR-gamma that is secreted from fat cells to affect those other tissues.

Identification of a Novel Factor Produced by Fat Cells in Mice: Resistin

PPAR-gamma receives signals from outside the cell and then induces changes in whether various genes are turned on or off ("gene expression"). Dr. Lazar hypothesized that the insulin-sensitizing drugs, through interaction with PPAR-gamma, may dampen the activity of a gene in fat cells involved in insulin resistance. Thus, to identify new adipocyte molecules that mediated insulin resistance, Dr. Lazar's laboratory looked for genes that were turned off by this class of insulin-sensitizing drugs in mice. They identified a gene that was suppressed in adipocytes by these drugs and named it "resistin" for its role in resistance to insulin. Mouse resistin is specifically produced and secreted by adipocytes. Resistin's secretion from the fat cells into the blood circulation is consistent with its potential role in affecting insulin resistance of other body tissues. In their study, Dr. Lazar and his colleagues showed that levels of resistin in the blood were increased in obese mice, including mice with a genetic mutation conferring obesity, and mice that were obese from having been fed a high-fat diet. Increasing levels of circulating resistin, by injecting resistin protein into mice, promoted insulin resistance. In contrast, decreasing levels of circulating resistin in mice, by injecting a factor that "neutralized" resistin protein, improved insulin sensitivity and decreased levels of blood glucose.

Further research in Dr. Lazar's laboratory revealed that resistin was a member of a novel family of secreted proteins in rodents and humans, and increased understanding of resistin's protein chemistry. Using genetic manipulations, Dr. Lazar and his colleagues showed that chronically high levels of resistin in mice of normal weight led to insulin resistance. By generating mice that lacked resistin (through genetic manipulations), Dr. Lazar and his colleagues found that mice without resistin were protected against insulin resistance. These genetic findings were confirmed by similar research in a number of laboratories. The results of these studies all nicely demonstrated that resistin was potentially an important regulator of insulin sensitivity and a promising candidate to link obesity to insulin resistance and diabetes.

Differences Emerge Between Mouse and Human Resistin

While research in mice provided important discoveries about the role of resistin, studies of resistin in humans were not so clear. A number of laboratories investigated whether high levels of resistin were linked to diabetes, obesity, and insulin resistance in people. Among the first published reports, many studies described a correlation between increased resistin levels and metabolic problems in humans. However, there were also studies that did not observe a correlation. More recent studies of resistin in humans that used larger population samples more convincingly link elevated levels of resistin to increased risk of type 2 diabetes. These studies also identify genetic variations associated with high resistin levels that correlate with insulin resistance in specific populations. These studies are providing further evidence that resistin, if not a causative factor, may be at least a biomarker of type 2 diabetes. Additional studies, including research from Dr. Lazar's team, show correlations between resistin levels and coronary atherosclerosis and increased risk of coronary artery disease. Other studies have shown that resistin levels are reduced by statin drugs and that very high resistin levels are correlated with a marked increase in the likelihood of heart failure.

So the studies increasingly suggest that resistin may be a factor in human metabolic diseases, including diabetes. But, not long after the discovery of resistin, another confusing issue arose: although mouse resistin is produced exclusively in white adipose tissue, several laboratories around the world (including Dr. Lazar's) found that human resistin is produced by cells of the body's immune system—known as monocytes and macrophages. This raised an interesting question:

Might the human resistin found in immune system cells have a similar metabolic function as the mouse resistin found in fat cells? Around this time, other researchers reported that adipose tissue is surprisingly not a pure population of fat cells, but contains macrophages as well, and that inflammation—a response of the immune system—is linked to metabolic conditions, like obesity and diabetes. By linking fat tissue and the immune system, these discoveries suggested that mouse and human resistin could be functioning in a similar manner despite their different cellular origins.

Human Resistin Links Inflammation and Metabolic Disease

To test whether inflammation affected levels of resistin in humans, Dr. Lazar and his colleagues injected healthy volunteers with a molecule that stimulates inflammation and a strong immune response. Researchers had previously noted that during this induced inflammatory state, people become temporarily insulin-resistant. Dr. Lazar's team measured levels of resistin following the injection and observed that resistin levels markedly increased in the stimulated inflammatory state. This and additional studies of the relationship between resistin and inflammation suggest that, in humans, inflammation is characterized by increased resistin levels.

Can macrophage-derived human resistin exacerbate metabolic disease, though? To answer this, Dr. Lazar and his colleagues turned their studies back to mice. This time they developed mice that were genetically engineered so that they would only produce the human version of resistin, not the native mouse resistin, and would only produce it in their macrophages, not in fat cells. The scientists compared these mice with littermates that didn't have any resistin to determine whether it is harmful to have macrophage-produced resistin.

Dr. Lazar and his laboratory found that, after 2 weeks of a high-fat diet, the mice carrying the human version of resistin were glucose intolerant and resistant to insulin—hallmarks of type 2 diabetes. Therefore, macrophage-derived human resistin contributed to insulin resistance in these mice. In addition, the expression of genes associated with inflammation was increased in the adipose tissue of these mice, indicating that resistin's ability to promote inflammation contributes to its role in insulin resistance. These results demonstrate that macrophage-produced human resistin promoted insulin resistance in mice fed high-fat diets, much like adipocyte-produced mouse resistin did in normal mice. These results also indicate that human resistin is an important link between inflammation induced by obesity and insulin resistance.

Having demonstrated similarities between mouse resistin and human resistin, Dr. Lazar plans to continue to elucidate the role of resistin in humans and its relationship with obesity, inflammation, and insulin resistance. He noted that the mice that harbor the human version of resistin could provide a model for testing potential therapies to block the actions of human resistin that lead to glucose intolerance and insulin resistance. In addition, scientists in Dr. Lazar's laboratory have created a mouse model that mimics the expression of human resistin even more closely. While in the first mouse model the macrophages continually produce human resistin, in the new mouse model human resistin production is responsive to inflammatory stimuli and is not continually produced. Future studies will include efforts to understand the role of human resistin in obesity and diabetes; cardiovascular disease, including atherosclerosis and heart failure; and inflammatory diseases, such as arthritis. Dr. Lazar hopes to determine whether resistin is a biomarker of and/or a potential risk factor for these conditions. Research from Dr. Lazar and his colleagues has provided great insight into the molecular links between inflammation, obesity, and metabolic diseases. This knowledge could promote the development of therapies to ameliorate the harmful effects of obesity, including insulin resistance and type 2 diabetes.

The Gould Family

Dedicated to Participating in Research To Be Part of a Cure for Type 1 Diabetes

The Gould family. Back row, left to right: Sam, Patrick, Ellen, Dave, Andrew, and Nicholas. Front row, left to right: Maggie, Annie, Sarah, and Oliver.
Photo credit: Amy McIntyre

Dave and Ellen Gould of Nashville, Tennessee, have eight children ranging in age from 2 to 17. Within the last 5 years, four of their children have been diagnosed with type 1 diabetes. Even though their lives are busier than most people can imagine, the Goulds make time not only to participate in clinical research studies, but also to tell others about the importance of research toward combating type 1 diabetes and finding a cure.

Their passion and dedication was evident this past summer, when Ellen testified in Congress at a hearing held in conjunction with the Juvenile Diabetes Research Foundation's 2009 Children's Congress. In her testimony, Ellen related how, on a Saturday morning several months earlier, the family was awakened by then 12-year-old son, Sam, who had collapsed in his room, incoherent, because of a dangerously low blood sugar level. "It took us 20 minutes to get him back to normal," Ellen said. "But what happens the next time if we don't hear him? As their mother, I just want to reach out and make it better—but I can't. I can't cure this disease; I can't make it better for my kids. I need help. Finding a cure means everything to my family, and we are willing to be part of the solution."

"Finding a cure means everything to my family, and we are willing to be part of the solution," said Ellen.

To that end, the Goulds are participating in NIDDK's Type 1 Diabetes TrialNet, an international network of researchers exploring new strategies to prevent, delay, and reverse type 1 diabetes. TrialNet is also supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*.

"There are a lot of smart people working on a cure for this disease," Dave said in a later interview. "I'm an optimist. I believe a cure is coming, and if my family can help speed it up a bit by being part of an important study, all the better."

"Diabetes Is Part of Our Family"

Type 1 diabetes is a chronic disease in which the body's immune system launches a misguided attack and destroys the insulin-producing cells of the pancreas. People with the disease need daily administration of insulin, either by injection or with a pump, and must monitor their blood sugar levels vigilantly.

"Diabetes is part of our family," Ellen said. "We're constantly filling prescriptions, scheduling doctors' appointments, filling out forms for school and various

activities, educating others—and making sure our kids are safe," she added.

For the Goulds, a life dominated by type 1 diabetes started 5 years ago when their oldest son, Patrick, then 12 years old, was diagnosed with the disease.

"I was watching him lose weight, and as a mother, I knew something was wrong," Ellen said. "I even asked Dave, 'do you think it could be diabetes?'"

As fate would have it, the family was on vacation when Ellen came upon a 1974 edition of *Life Magazine* at a flea market. The cover story just happened to be "Does Your Child Have Diabetes?" It was all the impetus she needed. As soon as the vacation ended, Ellen brought Patrick to their pediatrician and a blood test revealed that he had the disease. "Patrick's diagnosis came as a complete shock," Ellen said. "There's no history of diabetes in Dave's or my families."

Eighteen months later, their daughter Sarah, then 6 years old, began losing weight and urinating frequently at night. She too was diagnosed with type 1 diabetes. "Sarah took it very cavalierly, just like a trooper," says Ellen. But when Ellen and Dave told Patrick of his sister's diagnosis, "he just broke down and cried. Since having been diagnosed, Patrick had always dealt with his diabetes well and never really complained. But at that moment we knew how bad it was for him," said Ellen.

Shortly after Sarah was diagnosed, the family's endocrinologist told them about TrialNet, in which researchers were looking for children whose siblings had type 1 diabetes to see if other children in the family were at risk for developing the disease.

Dave said that at first he and Ellen didn't want to have their other children screened for the disease. "We just didn't want that cloud hanging over our heads," Ellen added. However, the more they thought about it, the more they began to realize that "maybe we can learn something from this. We also felt strongly that we needed to be part of this search for a cure, and the more we thought about it, the more enthusiastic we became," said Dave. It was through a TrialNet screening that the Goulds learned that then 10-year-old Sam also had type 1 diabetes.

Participating in a Clinical Trial To Prevent Type 1 Diabetes

A clinical trial being conducted by TrialNet is building on the results of a previous NIDDK-supported clinical trial, called the Diabetes Prevention Trial-Type 1 (DPT-1). The DPT-1 studied whether injected or oral insulin administration could prevent or delay type 1 diabetes in persons at high or moderate risk for the disease. While the DPT-1 did not find an overall protective effect of injected or oral insulin, a subset of trial participants who had higher levels of a predictive marker of the disease (insulin autoantibodies) seemed to benefit from oral insulin treatment, though this result was not definitive. TrialNet is now building on these observations, and has launched a clinical trial to determine if oral insulin therapy could prevent the disease in people with elevated insulin autoantibodies.

Through a TrialNet screening, the Goulds learned that 4-year-old Oliver had elevated levels of insulin autoantibodies, which made him eligible to enroll in the TrialNet oral insulin prevention study. The Goulds enrolled Oliver in the study, which randomly assigns participants to receive either an insulin pill or a placebo (inactive pill without insulin). Those participating in the trial do not know whether they are getting the insulin or the placebo. This randomization allows researchers to compare the two groups to determine if oral insulin could prevent or delay the development of type 1 diabetes.

Oliver has since developed type 1 diabetes—the fourth of the Gould's children to be diagnosed with the disease—and, until the study is over, the family will not know whether he received insulin or placebo. "When we decided to enroll Oliver in the study,

friends would ask, 'if you don't know whether he's receiving insulin or placebo, why did you enroll him?'" said Dave. "Ellen's and my response to them is: that's what research is. You have to be willing to accept that when you get into a study like this." He quickly added that, "We would be ready, willing, and able to do it all again. The best thing about TrialNet is that it's helping all of us move closer to preventing or delaying type 1 diabetes."

Likewise, significant knowledge will be gained no matter the outcome of the trial—it is only through a rigorous clinical trial that researchers definitively learn which therapies work and which ones don't. When effective therapies or preventative approaches are found, other patients and people at risk can benefit from them. If a potential intervention turns out to be ineffective, then scientists know to explore other avenues to find therapies that work. It is thanks to the dedication of the Goulds and other families that this important new knowledge can be gained.

"When we decided to enroll Oliver in the TrialNet study, friends would ask, 'if you don't know whether he's receiving insulin or placebo, why did you enroll him?'" said Dave. "Ellen's and my response to that is: that's what research is. You have to be willing to accept that when you get into a study like this." He quickly added that, "We would be ready, willing, and able to do it all again."

Fortunately, the Gould's other four children—Maggie, Annie, Nicholas, and Andrew—so far have tested negative for early markers of type 1 diabetes. But that doesn't mean that they are not affected by the disease. According to Ellen, 3-year-old Annie asks "When I am I going to get diabetes?" and 2-year-old Maggie tries putting Patrick's glucose meter on her finger to test herself. Describing his perspective on this disease, 13-year-old Nicholas said, "I'm really glad I don't have it. I see what my brothers and sister have to go through every day. I try to help as best I can, but I'm worried about them."

But Dave lays claim to being the family's ultimate worry-wart. "Ever since Sam's low blood sugar episode, I'm up with every bump I hear during the night, checking their bedrooms."

Through another study being conducted by TrialNet—the Natural History Study—the Gould's four children who don't have type 1 diabetes will continue to be screened annually. For the four who do have the disease, the best news to date is that they are all doing well and show no signs of complications from the disease.

"When I was first diagnosed," Patrick said, "I got a note from someone in the Juvenile Diabetes Research Foundation, and it said 'Hang in there. There's a cure coming. Take as good care of yourself as you can; you're not going to have to do this much longer.' My message to others with type 1 diabetes is the same: There's a cure coming. Hang in there."

As for his mother's testifying in front of Congress with her urgent message for finding a cure for her children and all the others who must deal with type 1 diabetes every minute of every day, Patrick said: "She was awesome!"

For information about participating in Type 1 Diabetes TrialNet, please call 1-800-HALT-DM1 or visit *www.diabetestrialnet.org*

PATIENT PROFILE

Leon Gibbs

Participant in Diabetes Prevention Program Outcomes Study (DPPOS) Continues To Be Diabetes-Free

Leon Gibbs

About 12 years ago, a doctor friend noticed that Leon Gibbs, then in his late 50s, was putting on weight. Knowing that diabetes runs in Leon's family, the friend urged, "Why don't you sign up for a study I'm involved in, called the Diabetes Prevention Program (DPP)?"

Leon took his physician friend's advice, and as a result became one of 3,234 people selected from around the country to determine if a modest amount of weight loss due to diet and exercise can prevent or delay the onset of type 2 diabetes. The study also tested whether a diabetes drug, metformin, could prevent onset of the disease. Participants were selected from a variety of ethnic backgrounds and cultures. All were either overweight or obese adults, and all had elevated blood glucose levels, making them high-risk candidates for type 2 diabetes.

Leon admits that the program was a challenge for him. "I'm not the best exercise person," Leon says, and healthy foods, like salads, had never been a part of his daily diet. But he persevered. He lost weight and gained weight, but remained diabetes-free throughout the duration of the original study. He was so impressed with the program that when a follow-up study was offered, called the Diabetes Prevention Program Outcomes Study (DPPOS), he signed up without hesitation.

"I owe a lot of thanks to the original prevention program, as well as to the follow-up outcomes study," says Leon, who now, at age 69, remains diabetes-free, healthy and active. "Just recently a friend complimented me on how good I look," says Leon. "I told him about the program and how much better I feel as a result of taking part in it."

"I owe a lot of thanks to the original prevention program, as well as to the follow-up outcomes study," says Leon, who now, at age 69, remains diabetes-free, healthy and active. "Just recently a friend complimented me on how good I look," says Leon. "I told him about the program and how much better I feel as a result of taking part in it."

Building Knowledge on Past Research

The studies Leon participated in—the DPP and the ongoing DPPOS—have taken researchers a long way in establishing that lifestyle intervention, such as diet and exercise, can reduce the risk of type 2 diabetes. In fact, the results of the original DPP study, which began in 1998, were so overwhelmingly

positive they were announced a year earlier than scheduled.

The DPP study demonstrated that both lifestyle changes and metformin are effective at reducing the risk of diabetes—the lifestyle changes especially so. After 3 years, those who participated in the lifestyle intervention portion of the study reduced their risk for development of type 2 diabetes by 58 percent, and study participants who took metformin reduced their risk by 31 percent, compared with placebo.

Getting with the Program

When he entered the DPP study, however, Leon was none too happy when he learned that he had been selected for the lifestyle intervention group. "I really did not want to diet and exercise. I wanted to take the easy way out and do the pill," he now chuckles. But knowing that his mother's two sisters had type 2 diabetes (one of whom subsequently died from the disease), he decided that joining the study was a good idea.

As part of the DPP, Leon started walking in his neighborhood and, using a pedometer, began keeping track of how many steps he took in a day, as well as his daily caloric intake. The program offered cooking classes and classes on how to read labels when shopping for food. "It's been a good lesson," Leon says of the program. He adds that he now stays away from sodium and sodas, eats less meat and more salads, and says he feels a lot better for it. As Leon began feeling more confidence in the program and in himself, he says, "I gradually got the gumption to ask waiters when I eat out, 'what's the most heart-healthy thing on your menu?'"

But controlling his weight hasn't exactly been a straight-line process for Leon. After retiring in 1991 weighing 200 pounds, he and his wife of 45 years, Doris, purchased a retail franchise and worked the evening shift together. After closing the place at 9 p.m., they would go home and eat late, and according to Leon, not-so-healthy dinners. As far as his diet and exercise during that period, Leon admits "I went through a lot of failure between 1991 and 1997." He entered the DPP 31 pounds heavier than he had been at "retirement." Even during the studies, his weight has continued to fluctuate.

Life-long Lessons

Leon jokes with family and friends that ever since he was in the DPP, and now the DPPOS follow-up study, he's probably lost 1,000 pounds—"and luckily I've only regained 995 of it back," he laughs. It makes for a good line, but there is clearly more to Leon's story. Despite the fact that Leon says he has gone through several periods of extreme enthusiasm followed by setbacks after he loses weight, the lessons he has learned from both of these studies obviously have stuck with him.

Two years ago, Leon bought a bike and began using it. He also walks 3 to 5 days a week and in the past year, his weight has gone from 242 pounds down to 214 pounds. To keep himself motivated and active, and being an avid golfer, he recently bought himself a new set of clubs. "My waistline has gone from 46 to 42 inches; I can do more now that I feel lighter," says the 6-foot-tall Leon. "And my wife and I have since sold our franchise, and as a result, I don't eat anything after 5 p.m. anymore," he says.

Leon continues to stay active in the DPPOS study. At visits he provides blood and urine samples, is administered an electrocardiogram to test his heart function, has his weight and height tracked and is asked if there have been any changes in his life since he was last seen. "Now they're also asking things that relate to your mental state, things like 'Are you more or less happy with your life?'" Leon says.

Study program staff also discovered that Leon had high blood pressure, as well as some high cholesterol levels. He appreciates that the study staff always send his medical records to his primary care physician

PATIENT PROFILE

and other doctors that he sees. "It's a very effective and efficient operation and takes a lot of redundancy out of my health care," he adds.

"From time to time the study folks send us literature on how to stay healthy," says Leon. But the thing he likes best are the booster classes, where he gets to share his experiences with others who are going through the same experience of having to diet and lose weight to stay healthy.

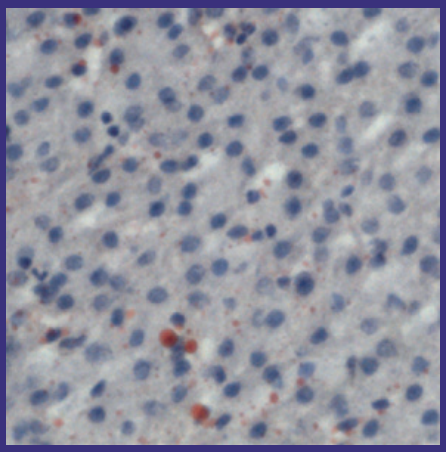
"I've learned a great deal about how to modify my eating habits, but it's hard to keep the weight off at my age," Leon adds, "that's why I like talking to people who are struggling with the same issues that I'm struggling with."

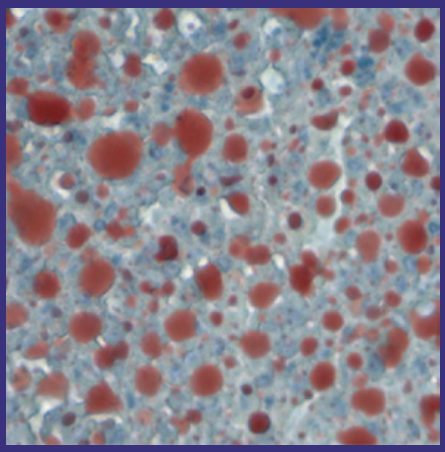
Leon has become a strong advocate of DPP and DPPOS, to the point where he has brought his wife to some of the classes the study offers on eating healthily. He even promotes healthy lifestyle changes to his 42-year-old son and his wife. "Unfortunately," he says, "they don't take my advice too seriously, but maybe one day they'll remember what I've told them."

Knowing how hard it is to lose weight, Leon says "You've got to change your mental attitude and be more mindful of what you put in your body. You've got to do everything you can to stay active. Keep moving. The rewards are worth it."

"You've got to change your mental attitude and be more mindful of what you put in your body. You've got to do everything you can to stay active. Keep moving. The rewards are worth it."

Though his blood glucose numbers "are still on the watch list, I remain diabetes-free," Leon says proudly.





Nonalcoholic fatty liver disease, a condition that resembles alcoholic liver disease but occurs in patients who drink little or no alcohol, is an increasingly important cause of liver failure and occurs in a high proportion of persons with diabetes. The disease is associated with overweight and obesity, and abnormal fat in the liver is also associated with the metabolic problem of insulin resistance. NIDDK-supported research highlighted in this chapter sheds new light on the effects of a high-fat diet during pregnancy on liver disease in offspring. For example, a recent study in non-human primates suggests that a chronic high-fat diet results in a significantly increased risk of fetal fatty liver disease that persists after birth. One striking example of increased fatty liver disease risk in offspring comes from examining the amount of fat stored in the liver cells from offspring of animals fed a low-fat diet (left) and a high-fat diet (right). The liver cells of offspring from animals fed a high-fat diet contained significantly greater amounts of fat (stained red) compared to those fed a low-fat diet, indicative of fatty liver disease development. Recognition of the role of the prenatal environment in the genesis of fatty liver suggests a potential approach to prevent the disorder. In addition to studies in animals, the NIDDK's Nonalcoholic Steatohepatitis (NASH) Clinical Research Network recently conducted two clinical trials that examined nonalcoholic fatty liver disease in children and adults. This chapter contains additional information about the impact of high-fat diets and other recent research on obesity and its associated diseases.

Obesity

Obesity has risen to epidemic levels in the U.S. Individuals who are obese may suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. Obesity is a strong risk factor for type 2 diabetes, fatty liver disease, and many other diseases and disorders within the NIDDK's mission.

Approximately one-third of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1,2] Moreover, while obesity and overweight have risen in the population in general, the greatest increases observed over approximately the past 2 decades have been in the prevalence of extreme obesity; those who are severely obese are most at risk for serious health problems.[3] Levels of childhood overweight and obesity have also escalated in the past several decades. Obesity affects approximately 16 percent of children and teens ages 2 through 19.[1,4,5] These children are at risk for developing serious diseases both during their youth and later in adulthood. Overweight and obesity also disproportionately affect racial and ethnic minority populations, and those who are socio-economically disadvantaged.

The increased prevalence of obesity in the U.S. is thought to result from the interaction of genetic susceptibility with behavior and factors in the environment that promote increased caloric intake and sedentary lifestyles. Thus, the NIDDK supports a multi-dimensional research portfolio on obesity, ranging from basic studies to large clinical trials. These studies have led to many important advances in our understanding of both the causes of obesity and in potential interventions for prevention and treatment. For example, researchers recently discovered the existence of brown fat tissue in adults, a finding with potential therapeutic implications. This metabolically active tissue burns fat molecules to generate heat, and in humans was once thought to exist only in infants. Other studies are looking at how the nutrients we eat may signal the brain to indicate fullness. A study in rodents revealed that a type of fat secreted into the blood from the small intestine in response to a high-fat meal may be linked to the ability to suppress appetite. Scientists are continuing to investigate the origins and development of fat cells with the goal of developing new therapies to combat obesity and type 2 diabetes. Other research includes observational studies to evaluate the risks and benefits of bariatric surgery as a treatment for severe obesity, as well as studies of behavioral and environmental interventions to reduce obesity in children and adults.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter. To help bring the results of research to the public and health care providers, the NIDDK also sponsors education and information programs. Given the importance of the obesity epidemic as a public health problem, and its relevance to the mission of the NIDDK, the Institute continues to play a leading role in the NIH Obesity Research Task Force. Co-chaired by the Directors of the NIDDK and the National Heart, Lung, and Blood Institute, the Task Force includes representatives from these and numerous other NIH Institutes, Centers, and Offices. With extensive input from external scientists and the public, the Task Force developed the *Strategic Plan for NIH Obesity Research*, published in 2004. In light of the many discoveries in the past several years, NIH scientists are currently in the process of updating the *Strategic Plan* and will be receiving input from

[1] *Statistics Related to Overweight and Obesity. http://win.niddk.nih.gov/statistics/index.htm*

[2] *Ogden CL, et al: JAMA 295: 1549-1555, 2006; Ogden CL, et al: NCHS Data Brief 1: 1-8, 2007.*

[3] *Flegal KM, et al: JAMA 288: 1723-1727, 2002; Flegal KM and Troiano RP: Int J Obes Relat Metab Disord 24: 807-818, 2000; Freedman DS, et al: JAMA 288: 1758-1761, 2002.*

[4] *Ogden CL, et al: JAMA 299: 2401-2405, 2008.*

[5] *For children and adolescents, this document uses the term obesity to refer to a BMI at or greater than the 95th percentile on growth charts (which are based on previous national surveys).*

external reviewers. Through this broad spectrum of research efforts, the NIDDK will continue to augment understanding of obesity and develop and test prevention and treatment approaches to improve health.

GENETICS OF OBESITY

Multiple Genetic Variations Linked to Overall Body Fat and Fat Distribution: By analyzing DNA from thousands of individuals, researchers have identified variations in multiple regions of the genome associated with common measures of obesity regarding total body fat and fat distribution. Although factors such as poor diet and lack of exercise are important contributors to obesity, genetic factors also help determine whether or not an individual is likely to become obese in a given environment. Past research on families and twins demonstrated that genetic factors account for much of the variation in body mass index (BMI), a common measure of body "fatness," across a population. In addition to BMI, other important measures of obesity, including waist size and the ratio of waist size to hip size, are believed to have a genetic component as well. To identify genetic factors associated with measures of obesity, an international consortium of researchers supported by NIDDK and others analyzed data from several, large-scale genome-wide association studies (GWAS). In GWAS, scientists scan the genomes of many individuals to look for genetic variations associated with a particular trait or condition, such as obesity.

Previous studies had identified common variants in two gene regions—or loci—that are believed to influence BMI. For one study of combined GWAS data, researchers looked for genetic variations associated with BMI regulation in more than 32,000 individuals of European ancestry. From this analysis, they uncovered genetic variations in or near six previously unreported gene loci that were reproducibly associated with adult BMI. Of the six BMI-related loci, five contain genes that are highly active in the region of the brain that regulates hunger, thirst, body temperature, fatigue, and circadian rhythms. These findings are consistent with the multiple possible roles of the central nervous system in controlling body weight, including regulation of appetite, energy expenditure, and other behavioral aspects.

Along with BMI, fat distribution—in particular, abdominal fat—has emerged as an important predictor of the risk of developing obesity-related disorders such as type 2 diabetes and heart disease. In a second analysis of data from several GWAS, scientists identified genetic variations that are associated with waist circumference or waist-to-hip ratio as measures of abdominal fat distribution. In addition to identifying some of the variants already associated with BMI, this study found novel variants in two loci that were strongly associated with waist size. One of the loci is near a gene called *TFAP2B*, which codes for a protein that is involved in regulating the accumulation of fat in fat cells. An additional variant near a third gene—*LYPLAL1*—was found to be associated with the waist-to-hip measure of fat distribution in women, but not in men. These results complement the findings of genetic variations associated with BMI, and identify some of the genetic components that regulate fat distribution in obesity, potentially identifying the genetic contributors to a subgroup at high risk for obesity-related diseases.

These two analyses of large, genome-wide studies have identified a number of novel genetic factors that are associated with measures of obesity. In most cases, further experiments will be needed to determine or confirm which genes—within the regions marked by the identified variants—have causal effects on obesity, and the mechanisms by which they contribute to obesity. Nonetheless, identification of these genes and gene loci provides new insights into the biological processes that regulate body weight and fat distribution, which may lead to the development of new therapeutic strategies for obesity.

Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, Berndt SI, Elliott AL, Jackson AU, Lamina C, Lettre G, Lim N, Lyon HN, McCarroll SA, Papadakis K, Qi L, Randall JC, Roccasecca RM, Sanna S, Scheet P, Weedon MN, Wheeler E, Zhao JH, Jacobs LC, Prokopenko I, Soranzo N, Tanaka T, Timpson NJ, Almgren P, Bennett A, Bergman RN, Bingham SA, Bonnycastle LL, Brown M, Burtt NP, Chines P, Coin L, Collins FS, Connell JM, Cooper C, Smith GD, Dennison EM, Deodhar P, Elliott P, Erdos MR, Estrada K, Evans DM, Gianniny L, Gieger C, Gillson CJ, Guiducci C, Hackett R, Hadley D, Hall AS, Havulinna AS, Hebebrand J, Hofman A, Isomaa B, Jacobs KB, Johnson T, Jousilahti P, Jovanovic Z, Khaw KT, Kraft P, Kuokkanen M, Kuusisto J, Laitinen J, Lakatta EG, Luan J, Luben

RN, Mangino M, McArdle WL, Meitinger T, Mulas A, Munroe PB, Narisu N, Ness AR, Northstone K, O'Rahilly S, Purmann C, Rees MG, Ridderstråle M, Ring SM, Rivadeneira F, Ruokonen A, Sandhu MS, Saramies J, Scott LJ, Scuteri A, Silander K, Sims MA, Song K, Stephens J, Stevens S, Stringham HM, Tung YC, Valle TT, Van Duijn CM, Vimaleswaran KS, Vollenweider P, Waeber G, Wallace C, Watanabe RM, Waterworth DM, Watkins N; Wellcome Trust Case Control Consortium, Witteman JC, Zeggini E, Zhai G, Zillikens MC, Altshuler D, Caulfield MJ, Chanock SJ, Farooqi IS, Ferrucci L, Guralnik JM, Hattersley AT, Hu FB, Jarvelin MR, Laakso M, Mooser V, Ong KK, Ouwehand WH, Salomaa V, Samani NJ, Spector TD, Tuomi T, Tuomilehto J, Uda M, Uitterlinden AG, Wareham NJ, Deloukas P, Frayling TM, Groop LC, Hayes RB, Hunter DJ, Mohlke KL, Peltonen L, Schlessinger D, Strachan DP, Wichmann HE, McCarthy MI, Boehnke M, Barroso I, Abecasis GR, and Hirschhorn JN, for the Genetic Investigation of ANthropometric Traits Consortium: Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 41: 25-34, 2009.

Lindgren CM, Heid IM, Randall JC, Lamina C, Steinthorsdottir V, Qi L, Speliotes EK, Thorleifsson G, Willer CJ, Herrera BM, Jackson AU, Lim N, Scheet P, Soranzo N, Amin N, Aulchenko YS, Chambers JC, Drong A, Luan J, Lyon HN, Rivadeneira F, Sanna S, Timpson NJ, Zillikens MC, Zhao JH, Almgren P, Bandinelli S, Bennett AJ, Bergman RN, Bonnycastle LL, Bumpstead SJ, Chanock SJ, Cherkas L, Chines P, Coin L, Cooper C, Crawford G, Doering A, Dominiczak A, Doney AS, Ebrahim S, Elliott P, Erdos MR, Estrada K, Ferrucci L, Fischer G, Forouhi NG, Gieger C, Grallert H, Groves CJ, Grundy S, Guiducci C, Hadley D, Hamsten A, Havulinna AS, Hofman A, Holle R, Holloway JW, Illig T, Isomaa B, Jacobs LC, Jameson K, Jousilahti P, Karpe F, Kuusisto J, Laitinen J, Lathrop GM, Lawlor DA, Mangino M, McArdle WL, Meitinger T, Morken MA, Morris AP, Munroe P, Narisu N, Nordström A, Nordström P, Oostra BA, Palmer CN, Payne F, Peden JF, Prokopenko I, Renström F, Ruokonen A, Salomaa V, Sandhu MS, Scott LJ, Scuteri A, Silander K, Song K, Yuan X, Stringham HM, Swift AJ, Tuomi T, Uda M, Vollenweider P, Waeber G, Wallace C, Walters GB, Weedon MN; Wellcome Trust Case Control Consortium, Witteman JC, Zhang C, Zhang W, Caulfield MJ, Collins FS, Davey Smith G, Day IN, Franks PW, Hattersley AT, Hu FB, Jarvelin MR, Kong A, Kooner JS, Laakso M, Lakatta E, Mooser V, Morris AD, Peltonen L, Samani NJ, Spector TD, Strachan DP, Tanaka T, Tuomilehto J, Uitterlinden AG, van Duijn CM, Wareham NJ, Hugh Watkins; Procardis Consortia, Waterworth DM, Boehnke M, Deloukas P, Groop L, Hunter DJ, Thorsteinsdottir U, Schlessinger D, Wichmann HE, Frayling TM, Abecasis GR, Hirschhorn JN, Loos RJ, Stefansson K, Mohlke KL, Barroso I, and McCarthy MI, for the Giant Consortium: Genome-wide association scan meta-analysis identifies three loci influencing adiposity and fat distribution. PLoS Genet 5: e1000508, 2009.

MOLECULAR REGULATORS OF METABOLISM

Metabolic Regulation by the Immune System: New research may help break the link between obesity and diabetes. Although overweight and obesity confer significant risk for developing type 2 diabetes, the precise mechanisms underlying the relationship between body fat and diabetes remain unclear. An accumulation of research over the last several years, however, implicates the chronic, low-grade inflammation that typically accompanies obesity in promoting insulin resistance, which is a precursor to type 2 diabetes. Earlier work found a type of immune system cell, the macrophage, is abundant in the fat tissues of obese mice (and humans). Macrophages are responsible for producing some of the chemical signals that trigger the inflammatory response. Now, new research finds that a different type of immune system cell—called $CD4^{+}Foxp3^{+}$ Regulatory T (Treg) cells—are found in the fat of lean animals, but not in those that are overweight. Experimentally depleting Treg cells from lean mice increased their resistance to insulin. Importantly, the researchers found that the Treg cells produce chemical signals that attenuate the inflammatory response when experimentally introduced into fat mice, helping restore the animals to a more normal metabolic state. This suggests that restoring normal immune regulatory signals in people at risk for type 2 diabetes may one day interrupt the development of insulin resistance and help to prevent the disease.

Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, and Mathis D: Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 15: 930-939, 2009.

Cells Self-Digest To Burn Fat for Energy When Starved: Scientists have discovered that cells use a 'self-destruction' pathway to burn fat for energy during times of nutrient starvation. When nutrients

are scarce, cells turn on metabolic pathways that mobilize stores of fat as a source of energy to carry out necessary cellular functions. Molecules of fat known as triglycerides (TGs), together with cholesterol, are stored in cellular compartments called "lipid droplets." The composition of lipid droplets is normally dynamic, where TGs are continuously broken down into their component parts—free fatty acids—and reassembled into TGs for storage depending on the cell's energy needs. During times of starvation, cells trigger the breakdown of TGs into free fatty acids, which serve as fuel for the cell.

In a recent study supported by NIDDK, scientists demonstrated that the breakdown of TGs during nutrient deprivation is mediated, in part, by a cellular process known as autophagy. Autophagy, which translates literally as "to eat oneself," is a complex metabolic process in which cellular components are packaged and delivered to cellular depots called lysosomes. Material destined for destruction in the lysosome is broken down and recycled for other purposes. In addition to the previously known role for autophagy in general cellular "housekeeping," it now appears that autophagy also plays an important role in breaking down fats in the liver during starvation. To explore the link between autophagy and processing of fats, the scientists used pharmacologic agents and genetic engineering to block autophagy in laboratory-grown liver cells or in the livers of mice, and found that TGs and lipid droplets accumulated. In addition, by examining the cells microscopically under various experimental conditions, the researchers found that components of the autophagy machinery associate with lipid droplets and deliver the droplets to the lysosome for destruction. In mice, the autophagy-mediated destruction of lipid droplets was activated following prolonged nutrient starvation. When mice are fed a high-fat diet, on the other hand, the excess fat appears to inhibit the autophagy pathway and, in a cyclical fashion, further increase fat accumulation. This is of clinical importance, as the decrease in autophagic function with aging may contribute to the accumulation of fat in the liver associated with a constellation of health problems referred to as the metabolic syndrome. Therapeutic strategies that stimulate autophagy to promote the breakdown of fats in the liver may, therefore, be a useful approach for preventing metabolic syndrome.

Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, and Czaja MJ: Autophagy regulates lipid metabolism. Nature 458: 1131-1135, 2009.

Controlling Metabolism with Genes: A new study has found that a protein called Jhdm2a is able to control body weight in mice by turning on or turning off genes involved in metabolism. Jhdm2a does this by modifying a protein that packages DNA. In a cell, DNA molecules are wrapped around proteins called histones. Changing the way that the DNA molecule wraps around a histone, or the structure of the histone itself, can change the way genes are turned on or off. One way that this DNA-protein interaction can change is through chemical modification of a histone. One factor known to chemically alter histones is Jhdm2a. Previously, this factor was shown to have an important role in a different process: the development of sperm in mice. Interestingly, when examining an experimental mouse model that lacked Jhdm2a, a group of scientists recently noticed that these mice became obese in adulthood, as compared to normal animals. Turning their attention to a potential role for Jhdm2a in body weight, the researchers designed further experiments that revealed that Jhdm2a protein levels are high in organs involved in metabolism, such as brown fat tissue and muscle. These observations led the scientists to speculate that Jhdm2a plays an important role in regulating metabolism.

To better understand the role of Jhdm2a in controlling metabolism, the researchers decided to conduct additional studies of mice lacking this protein. Without Jhdm2a, the mice accumulated large droplets of fat in their fat tissue cells as well as in other organs, such as liver and muscle, and they also had high levels of fat in their blood despite eating a normal diet. These results suggested that a lack of Jhdm2a results in abnormal fat metabolism and obesity. Subsequent experiments revealed that the absence of Jhdm2a protein led to the gene *Ppar-alpha* being turned off; this gene plays a key role in fat metabolism in skeletal muscle. The scientists also determined that another gene important in controlling energy balance, *Ucp1*, is not activated normally in the brown fat of mice lacking Jhdm2a protein. One metabolic result of this defect is that the mice without Jhdm2a were unable to maintain body temperature as well as normal mice in cold temperatures, likely because their brown fat

could not burn energy to generate heat as efficiently. Additional studies showed that the absence of Jhdm2a protein resulted in cells failing to turn on other factors necessary for *Ucp1* activity. Given these results, the researchers proposed that Jhdm2a mediates certain cell signaling pathways based on systemic energy demands, and is thus a key regulator of energy balance and body weight. Although the precise manner by which Jhdm2a controls metabolism is not yet completely understood, this study may lead to novel approaches for obesity prevention or treatment.

Tateishi K, Okada Y, Kallin EM, and Zhang Y: Role of Jhdm2a in regulating metabolic gene expression and obesity resistance. Nature 458: 757-761, 2009.

SMRT Mice—Discovering Molecular Controls of Metabolism: Scientists have discovered that a protein called SMRT, which is involved in turning various genes off, plays a major role in regulating metabolism and fat cell formation. Metabolism is a complex and carefully regulated process that involves turning numerous genes on or off (called "gene expression"). Nuclear hormone receptors (NHRs) are proteins that moderate a wide variety of biological processes by controlling gene expression, and are known to have critical roles in regulating metabolism and development. NHRs control gene expression, in part, by interacting with proteins called "corepressors," which prevent genes from being turned on. However, the precise function of NHR-interacting corepressors, and the role corepressors may play in metabolism, is not well understood.

The SMRT protein is a corepressor known to be involved in cellular and organ development. It was believed to require interaction with NHRs for at least some of its activities. Reducing or eliminating levels of SMRT in mouse models results in brain and heart defects and death during embryonic development. To further examine the role of SMRT and its interaction specifically with NHRs, scientists generated a genetically-engineered mouse in which SMRT is present but unable to physically interact with NHRs. Interestingly, although these mice lived and had normally developed organs, they displayed a number of metabolic disorders. The mice had significantly decreased metabolic rates and developed factors predisposing them to type 2 diabetes, including high blood glucose levels and insulin resistance.

Moreover, the mice had significantly increased volumes of fat tissue due to an increased number of fat cells. Fat cells, or "adipocytes," are believed to be derived from stem cells known as fibroblasts. Under normal circumstances, a small fraction of mouse embryonic fibroblasts will develop into adipocytes when exposed in the laboratory to a combination of proteins or chemicals. When researchers isolated embryonic fibroblasts from the genetically-engineered mice, however, and treated these cells with a mixture of reagents known to induce conversion into adipocytes, nearly all the fibroblasts became mature adipocytes. More surprisingly, researchers discovered that embryonic fibroblasts isolated from the mice were able to spontaneously turn into adipocytes without exposure to the reagents. Additionally, the researchers found that cells with the altered SMRT proteins had increased levels of expression of key fat cell genes. These experiments led scientists to conclude that SMRT is a critical factor in determining whether an embryonic fibroblast will develop into a fat cell.

This research provides the first evidence that SMRT is required for metabolic balance. Likewise, SMRT also appears to regulate the development of adipocytes and fat tissue. These data suggest that various metabolic processes are influenced or controlled by NHR-SMRT interaction, and may provide future targets for pharmaceutical interventions to treat metabolic disorders.

Nofsinger RR, Li P, Hong SH, Jonker JW, Barish GD, Ying H, Cheng SY, Leblanc M, Xu W, Pei L, Kang YJ, Nelson M, Downes M, Yu RT, Olefsky JM, Lee CH, and Evans RM: SMRT repression of nuclear receptors controls the adipogenic set point and metabolic homeostasis. Proc Natl Acad Sci USA 105: 20021-20026, 2008.

GUT MICROBES, ADIPOSITY, AND OBESITY

Protein on Intestinal Cells Controls Use of Nutrients Digested by Microbes: Scientists have discovered how the intestines detect and absorb some of the nutrients—and the associated calories—that have

been processed from the diet by gut microbes. The ability of the human digestive system to metabolize nutrients is supplemented by the microbial community residing inside the intestines. This community provides an array of digestive functions, including breaking down dietary complex carbohydrates, which humans cannot digest on their own, into smaller molecules called short-chain fatty acids (SCFAs), which can readily be absorbed by the intestines and used as a source of energy. SCFAs resulting from microbial digestion are detected by a protein called Gpr41—a receptor that sits on the surface of cells lining the intestines. Yet, the details of how Gpr41 triggers cellular responses that regulate extraction of microbially produced nutrients, and how this might contribute to overall energy balance, were obscure.

To understand how Gpr41 detects and regulates use of SCFAs produced by intestinal microbes, NIDDK-supported scientists compared normal mice to genetically-engineered mice that lacked the Gpr41 protein. In addition to the genetic modifications, the scientists also manipulated the intestinal microbial communities of these mice by raising them either conventionally (with a full intestinal microbial community), under germ-free conditions (in which the intestine would be free of microbes), or initially germ-free followed by addition of two key microbes found in human intestine—*Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii*. Removing Gpr41 in mice that have either a full intestinal microbial community or just the two key microbes caused them to gain less weight (*i.e.*, extract fewer nutrients, and thus calories, from the same carbohydrate-rich diet as normal mice with Gpr41). The scientists demonstrated that this effect in Gpr41-deficient mice was associated with a decrease in production of a hormone called PYY, which controls gut motility. The lower levels of PYY hormone in Gpr41-deficient mice caused nutrients to pass through the digestive tract more quickly than in normal mice, resulting in less absorption of the SCFAs by the intestines and greater excretion from the body in waste.

The results of this study demonstrate that in normal mice with functioning Gpr41, detection of microbially produced SCFAs by this receptor causes a change in hormone levels that modulates how efficiently these nutrients are absorbed by the intestines and made available as an energy source. The microbial community in the intestine of obese mice is more efficient at converting carbohydrates to SCFAs and extracting energy from the diet. If Gpr41 functions similarly in humans, it could serve as a therapeutic target for decreasing nutrient absorption and thus reducing weight in obese individuals.

Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, and Gordon JI: Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci USA 105: 16767-16772, 2008.

Obesity Linked to Unique Mix of Intestinal Bacteria and Bacterial Genes: Scientists have discovered that bacteria that dwell in the human gut are associated with their host's obesity or leanness. This work provides clues to how knowledge of the gut microbial community might be used to counter human obesity. Bacteria that inhabit the gut—the gut microbiota—perform important functions, including breaking down food that could not otherwise be digested. Mouse studies have also suggested that bacterial diversity in the gut may influence whether animal "hosts" are lean or obese, based on differences in the efficiency of specific types of bacteria to extract energy (calories) from food.

In a recent study of obese and lean adult twin sets and their mothers, researchers studied the human microbiota using fecal samples to determine whether host obesity, genetics, or environment is associated with the bacterial composition of the microbiota. To determine which types of bacteria were present in the gut, the researchers analyzed DNA sequences in a particular gene common to all bacteria to identify sequence variations unique to each type. Comparisons revealed that the proportion of different types of bacteria in the guts of obese twins differed from that in the lean twins. *Actinobacteria* were more abundant than *Bacteriodetes* bacteria in the obese twins. Conversely, *Bacteriodetes* were more numerous in the lean twins. Obesity was also associated with significantly less bacterial diversity overall than leanness. Additional analysis revealed that the microbiota of family members are more similar in

bacterial composition than unrelated individuals. Surprisingly, the identical twins were not more similar in their gut microbes than fraternal twins, suggesting that composition of the gut microbiota is influenced more strongly by environmental factors than by an individual's genes. An analysis of bacterial genes represented in the "microbiome"—the combined DNA of the microbiota—found that although the precise composition of the types of bacteria in the gut differs among individuals, people share a "core microbiome" of common microbial genes harbored by the various bacteria. Additionally, comparison of non-core microbiome genes identified over 350 genes that were either enriched or depleted in the microbiomes of obese individuals. Among the genes enriched in the obese gut microbiome, many of which are involved in processing carbohydrates and other metabolic pathways, most were from *Actinobacteria* and others were from another group of bacteria, *Firmicutes*.

While this study does not demonstrate cause and effect—whether differences in human microbiota help cause obesity or leanness, or whether obesity or leanness leads to changes in gut microbes—earlier research has shown that the composition of gut microbiota can influence weight gain in mice. This study does demonstrate a significant link between obesity and the gut microbiome, including the identification of several hundred genes that represent biomarkers of unique gut bacterial activity in obese individuals. These biomarkers may lead to more personalized health care and potential probiotic interventions to modify the microbial content of the human gut.

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, and Gordon JI: A core gut microbiome in obese and lean twins. Nature 457: 480-484, 2009.

NEW INSIGHTS INTO FAT

Finding Where Fat Comes From—Research on Fat Cell Precursors: A recent study has shed new light on how fat cells, also known as adipocytes, develop. Understanding fat tissue biology and how adipocytes develop will help scientists develop potential new therapies to combat obesity and diabetes. These metabolic disorders have reached epidemic levels, and the resulting public health crisis necessitates an understanding of fat tissue function. Fat cell development is a fundamental process that has important biological impacts. Fat cells develop from an immature cell type (precursor cell). However, little is known about the identity of these precursors, when they become destined or committed to give rise to fat cells, and precisely where they are located. The precursor cells are believed to reside in a specialized part of fat tissue called the stromal-vascular (SV) compartment. For a precursor cell to develop into a mature adipocyte, the precursor cell must produce (or "express") a protein known as PPAR-gamma. Because PPAR-gamma plays an integral role in fat cell development, scientists used this protein as a tool to study the precursors and observe how precursor cells transformed into fat cells. To do this, researchers created a genetically-engineered mouse in which any cell that produces PPAR-gamma is permanently marked so that it—and if it divides, any cell derived from it—can be easily identified. By studying these mice shortly after birth, the researchers discovered that the majority of the adipocytes in the mice were derived from a pre-existing pool of cells that express PPAR-gamma. Subsequently, the scientists determined that fat cells in the young mice developed from precursor cells that are present prior to birth and can replicate, and are the primary source of large numbers of fat cells that develop during the first month of life.

Further experiments determined that the SV compartment within fat tissue contains a large number of PPAR-gamma-expressing cells that are able to divide and provide a source of precursor cells that in turn mature into adipocytes. These PPAR-gamma-expressing cells reside in tubelike structures that appear to be blood vessels within the SV compartment of white fat deposits. Detailed analysis revealed that the PPAR-gamma-expressing cells in these vessels represent a unique population within fat tissue and are biologically distinct from fully developed adipocytes. Interestingly, when these PPAR-gamma-expressing cells were isolated from the SV compartment in fat tissue and transplanted into other mice, the cells could be chemically induced to develop into mature adipocytes. This indicated that the PPAR-gamma-expressing cells isolated from the

fat tissue SV compartment have the unique ability to develop into adipocytes and serve as a source of fat cells. Collectively, these studies indicate that the interaction between fat tissue and the SV compartment could potentially provide targets for future therapies to treat obesity and diabetes. Understanding the process of adipocyte development can help identify future strategies to preempt disease and metabolic disorders.

Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD, and Graff JM: White fat progenitor cells reside in the adipose vasculature. Science 322: 583-586, 2008.

Calorie-Burning Fat Found in Lean Adults: New research has revealed that an energy-burning form of fat is active in adults—a finding that may open new avenues for efforts to combat obesity, a strong risk factor for type 2 diabetes. Unlike "white fat," which stores energy and comprises most body fat, another type of fat, called "brown fat," burns calories to help keep animals warm. In humans, it has been thought that brown fat is active only in infants. Now, using advanced imaging technology (PET-CT scans), a new study has found evidence that a significant portion of adults retain metabolically active brown fat. In this study, researchers detected substantial amounts of active brown fat in the neck region of adults. They also found some key differences among people. Women were more than twice as likely as men to have substantial amounts of this fat. Older people tended to have less brown fat, but being thinner was associated more with having brown fat, especially among older people—suggesting that brown fat may help protect against age-related weight gain. Interestingly, the researchers also observed that a person's brown fat changed with the outdoor temperature, with the most brown fat activity detectable in colder weather. This finding is consistent with two other research studies (funded in Europe) that were published at the same time, which showed that brown fat activity increased in people briefly exposed to cold. These clinical findings dovetail with recent insights in animal models into the molecular signals controlling the growth of brown fat. Together, these discoveries may help scientists develop therapeutic drug interventions to promote weight loss through increasing brown fat, or to exploit the finding that brown fat is activated by exposure to cold temperatures.

Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, and Kahn CR: Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360: 1509-1517, 2009.

Fat That Might Make Us Thin: A recent study has determined that a type of fat normally found in blood can help control hunger and food intake. Appetite is partially regulated by specialized regions of the brain so that energy intake (food calories) is balanced with energy expenditure. Hormones, nutrients, and other factors interact with the brain to mediate both short- and long-term feeding behavior. Fats circulating in the blood are also thought to help regulate appetite by interacting with the brain. However, it is unclear how this is accomplished.

In rats, scientists observed that a relatively abundant type of fat, called NAPE, is secreted into the blood from the small intestine after the animals are fed a meal containing high levels of dietary fats. Until this time, NAPE did not have a known physiological function. When the rats ate a meal containing a high level of fat, scientists observed a 60 percent increase in NAPE in the rats' blood. To make certain that the significant increase in NAPE was due to the dietary fat, the rats were injected with a solution of fat, glucose, or protein. Rats that were injected with fat had a 50 percent increase of NAPE in their blood while the animals injected with protein or glucose had no increase in NAPE levels. In addition, the elevated NAPE levels in the fat-injected animals persisted for 6 hours. To confirm the role of NAPE in appetite regulation, the researchers injected the rats with NAPE. Animals that were injected with different amounts of NAPE decreased their food intake accordingly. Rats that received a large amount of NAPE ate no food over a 12-hour period, whereas rats that were injected with a small amount of NAPE slightly decreased the amount they ate. Next, researchers examined the link between the brain and NAPE. Injecting NAPE directly into the brains of mice drastically reduced the animals' food intake for 12 hours. Additionally, the researchers found that NAPE circulating in the blood is able to enter the brain and accumulates in the hypothalamus, a part of the brain associated with regulating metabolism, digestion, and appetite. Furthermore, increased levels of NAPE in the blood of mice increased the activity of a specific kind

of neuron in the hypothalamus that controls hunger and food intake.

The ability of NAPE to control diet-induced obesity was highlighted by measuring NAPE production in rats that were fed a high-fat diet or their regular, lower-fat chow for 1 month. Subsequently, all the rats were given a high-fat meal, and then the amount of NAPE circulating in the blood was measured. Animals that had been fed the lower-fat diet had a significant increase in their plasma NAPE levels after eating the high-fat meal. Surprisingly, mice that had been fed the high-fat diet for a month were not able to increase their plasma NAPE concentrations following a high-fat meal. However, when the rats that consistently ate a high-fat diet were injected with NAPE, they significantly reduced their food intake. Finally, the researchers observed that infusions of NAPE also reduced body weight in the rats. These studies highlight the important link between NAPE and the ability to control hunger and food consumption in rodents. If this newly identified role for NAPE turns out to be similar in people, then this finding will have implications for a new strategy for treating diet-induced obesity.

Gillum MP, Zhang D, Zhang XM, Erion DM, Jamison RA, Choi C, Dong J, Shanabrough M, Duenas HR, Frederick DW, Hsiao JJ, Horvath TL, Lo CM, Tso P, Cline GW, and Shulman GI: N-acylphosphatidylethanolamine, a gut-derived circulating factor induced by fat ingestion, inhibits food intake. Cell 135: 813-824, 2008.

CONSEQUENCES OF EXCESS FAT

Maternal High-Fat Diet During Pregnancy Triggers Liver Disease in Offspring: A new study highlights a risk of liver disease resulting from exposure to excess fat and calories during fetal development. Childhood obesity has been correlated with the dramatic rise in incidence of type 2 diabetes and nonalcoholic fatty liver disease among youth in the U.S. Previous studies have shown that high-fat diets, obesity, and diabetes during pregnancy are associated with metabolic problems in the offspring. However, the mechanisms for these findings remain not well understood, and it can be difficult to distinguish between the effects of obesity and the effects of a diet that could lead to obesity. Current dietary guidelines for mothers with gestational diabetes include substituting fats for carbohydrates in the diet to help lower blood glucose levels. However, the results from this study suggest the importance of considering potential adverse effects of excess dietary fat when adjusting a diet.

Scientists recently explored the effects of a high-fat diet during pregnancy on liver disease in offspring, using an animal model in which some mothers were more susceptible to obesity and diabetes than others. These studies, done in non-human primates, suggest that a chronic high-fat diet results in a significantly increased risk of fetal fatty liver disease that persists after birth. This finding held true whether or not the mothers were themselves obese or had diabetes. Pregnant animals fed a high-fat, high-calorie diet produced offspring that had a three-fold increase in triglyceride fat in the liver. Furthermore, the offspring displayed evidence of increased liver stress during gestation, consistent with the development of fatty liver disease. Elevated levels of triglycerides persisted in the offspring following birth. Additionally, as the offspring grew, those from mothers fed a high-fat diet had a two-fold increase in percent body fat compared to offspring of mothers who ate a standard diet. Importantly, after female animals were consistently fed a high-fat diet for 4 years, switching them to a lower calorie, low-fat diet reduced fetal liver abnormalities in their subsequent offspring, even though some of the mothers remained obese and insulin-resistant. These studies suggest that a maternal high-fat diet may result in increased fat transfer to the fetus, and that unhealthy levels of fats in maternal blood (rather than diabetes or obesity) could potentially be the predominant cause of some future metabolic disorders in offspring. However, it is also possible that the health effects observed in offspring were the result of the high total calories fed to their mothers, rather than the percentage of calories from fat.

Fatty liver disease is an increasingly important cause of liver failure. Recognition of the role of the prenatal environment in the genesis of fatty liver provides a potential intervention to prevent the disorder. By shedding new light on dietary contributors to adverse health conditions, this study can direct future research efforts toward developing strategies to preempt disease.

McCurdy CE, Bishop JM, Williams SM, Grayson BE, Smith MS, Friedman JE, and Grove KL: Maternal high-fat diet triggers

lipotoxicity in the fetal livers of nonhuman primates. J Clin Invest 119: 323-335, 2009.

How Excess Lipid Leads to Cell Death: A recent study has shed new light on how fat causes cells to die, implicating a gene called *gadd7* and the molecule it encodes—a form of RNA. Cells react to extreme or prolonged stress, such as metabolic imbalances, physical injury, or improper protein folding, by activating pathways that lead to cell death. Lipids are normally stored in fat tissue that contains cells specifically designed for this function. When the amount of lipid exceeds the storage capacity of fat tissue, as seen in obese patients, lipid accumulates in locations other than fat tissue, such as the liver, kidneys, pancreas, or muscle. Improper lipid storage can lead to abnormal cell function, cell death, and organ failure, a condition called "lipotoxicity." Organ damage due to lipid storage in areas other than fat tissue is associated with the production of "free radicals"—highly reactive forms of oxygen molecules that cause damage to cells. Damage to cells by free radicals is known as "oxidative stress." Scientists have observed that feeding large amounts of saturated fat to cells isolated in the laboratory causes the cells to die due to oxidative stress. However, it is unclear precisely how excess fat leads to oxidative stress and cell death.

Researchers discovered that cells containing a defective version of the *gadd7* gene are resistant to cell death due to lipotoxicity when grown in liquids containing high levels of fat. To explore the molecular mechanisms underlying lipotoxicity, scientists generated mutant cells that could withstand excess saturated fat. These cells turned out to have a defect in the *gadd7* gene, and the scientists thus surmised that the normal form of *gadd7* must be involved in lipotoxicity. This gene was known previously to encode an RNA molecule, which it produced in response to exposure to hydrogen peroxide, a free radical. In the current study, when cells were incubated in high levels of fat, *gadd7* RNA levels increased in response to the lipotoxic environment and the subsequent generation of free radicals in the cell. If the cells were given antioxidants, both free radical and *gadd7* RNA levels were significantly reduced. The survival of the *gadd7* mutant cells in lipotoxic conditions was not due to an inability of the cells to take up fat from their environment. To confirm that *gadd7* plays a role in facilitating lipotoxic-induced cell death, the researchers created cells that produce levels of *gadd7* RNA significantly lower than normal. Reducing cellular levels of *gadd7* RNA led to protection against cell lipotoxicity when the cells were grown in the presence of high levels of fat. Furthermore, reducing *gadd7* RNA levels in the cells also led to a decrease in another characteristic cellular response to excess lipids—damage to the endoplasmic reticulum, a key component of the cell.

Taken together, these experiments demonstrate that *gadd7* RNA serves as a regulator for lipid-mediated oxidative stress and cell death. Although the precise manner by which *gadd7* affects lipotoxicity is not yet known, the data point to *gadd7* as a key mediator of cellular damage caused by oxidative stress. This is a unique function for an RNA molecule, and may serve as a potential target for therapies directed toward combating obesity-related diseases.

Brookheart RT, Michel CI, Listenberger LL, Ory DS, and Schaffer JE: The non-coding RNA gadd7 is a regulator of lipid-induced oxidative and endoplasmic reticulum stress. J Biol Chem 284: 7446-7454, 2009.

OBESITY, DIABETES, AND URINARY INCONTINENCE

Urinary Incontinence in Women Who Have Type 2 Diabetes and Are Overweight: Researchers have recently reported that urinary incontinence is highly prevalent among overweight and obese women with type 2 diabetes, and far exceeds the prevalence of other diabetes complications among this population. Using a self-report questionnaire, 2,994 women enrolled in the Action for Health in Diabetes (Look AHEAD) study indicated their frequency and type of incontinence within the last year. Analysis of the data revealed that weekly incontinence, experienced by 27 percent of participants, was reported more often than other diabetes-associated complications such as blood vessel changes in the eye, nerve pain, or protein in the urine. Furthermore, urinary incontinence affected non-Hispanic whites more than Asians or African Americans. Among the cohort, obesity was found to be the strongest modifiable risk factor for urinary incontinence. Another NIDDK-supported

study, the Program to Reduce Incontinence by Diet and Exercise (PRIDE), recently demonstrated that modest weight loss reduces urinary incontinence episodes in overweight and obese women who do not have diabetes. Ongoing research efforts by Look AHEAD investigators will reveal whether weight loss also reduces urinary incontinence among women with type 2 diabetes.

Phelan S, Kanaya AM, Subak LL, Hogan PE, Espeland MA, Wing RR, Burgio KL, DiLillo V, Gorin AA, West DS, Brown JS, on behalf of the Action for Health in Diabetes (Look AHEAD) Research Group: Prevalence and risk factors for urinary incontinence in overweight and obese diabetic women: Action for Health in Diabetes (Look AHEAD) study. Diabetes Care 32: 1391-1397, 2009.

RESEARCH ON BARIATRIC SURGERY

Evaluating the Safety of Bariatric Surgery: The Longitudinal Assessment of Bariatric Surgery (LABS) consortium conducted a multicenter, observational study to evaluate the 30-day safety outcomes in patients who underwent an initial bariatric surgical procedure. Approximately one-third of U.S. adults are considered obese based on their body mass index or BMI (a measure of weight relative to height); these individuals have increased risk for type 2 diabetes, coronary heart disease, stroke, fatty liver disease, certain types of cancer, and other diseases. Bariatric surgery is considered appropriate for those who are extremely obese (BMI of 40 or more) or those with a BMI of 35 or more who have significant obesity-related conditions, such as type 2 diabetes or sleep apnea. Approximately 6 percent of U.S. adults are extremely obese. Used as a treatment for extreme obesity, bariatric surgical procedures modify the digestive tract to limit the amount of food that can enter the stomach, decrease absorption of nutrients, or both. Currently, bariatric surgery appears to be the only intervention that consistently results in substantial and sustained weight loss in people who are extremely obese, and it has been linked to remission of type 2 diabetes, decreases in cardiovascular risk factors, and a significant reduction in mortality over time. Like most surgical procedures, however, bariatric surgery presents risks of complications and death that must be considered when deciding whether to undergo the procedure.

In this study, LABS-1, the consortium followed 4,776 patients who had bariatric surgery, from before their surgery through the first 30 days following surgery, to evaluate death and complication rates. All of the patients participating in the study were adults and were obese, and most had a BMI measurement reflecting extreme obesity. Similar to most populations undergoing bariatric surgery, the majority of the patients in the LABS-1 study were white and female. The study took place over 2 years at 10 medical centers located throughout the U.S., with one center coordinating data collection and analysis. Within 30 days of surgery, 4.1 percent of patients had at least one major adverse outcome, defined as development of blood clots in the deep veins of the legs or the pulmonary artery of the lungs, repeat surgeries, not being discharged from the hospital within 30 days, or death. Mortality rates were low: less than 1 percent (0.3 percent) of patients died within 30 days. The risk of complications varied depending on whether or not patients had certain health conditions prior to the surgery and how obese they were. Although the rate of adverse events also appeared to vary with the type of surgical procedure, differences in patient characteristics may have accounted for much of the variation in risk among the procedures. Further investigation may help clarify any such differences.

This evaluation highlights the level of short-term risks associated with bariatric surgery, an effective weight loss procedure that is increasingly popular as a treatment for extreme obesity. The safety of such surgery is an important consideration with risks examined in the context of long-term benefits. The LABS-1 study will help health care providers and patients make personalized decisions about the potential risks and benefits of bariatric surgery by taking into account a patient's characteristics. Another study being conducted by the LABS consortium, LABS-2, will follow a subset of the patients to gather longer-term data that will further inform decisions about the surgery.

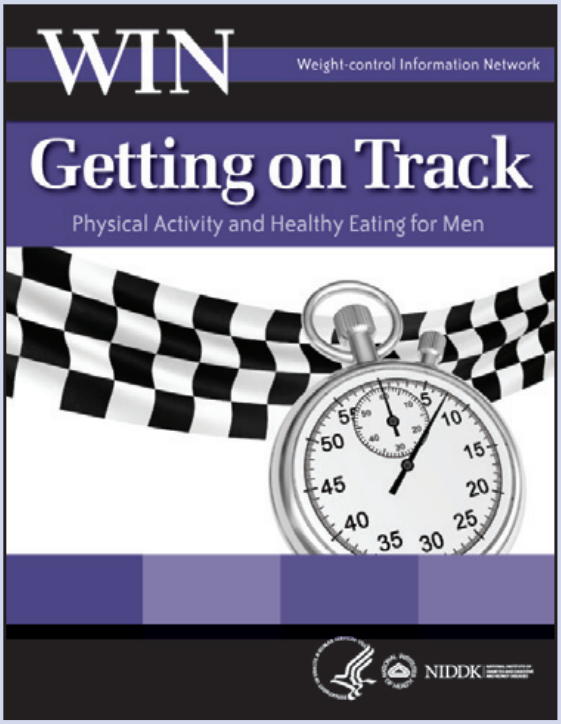
Flum DR, Belle SH, King WC, Wahed AS, Berk P, Chapman W, Pories W, Courcoulas A, McCloskey C, Mitchell J, Patterson E, Pomp A, Staten MA, Yanovski SZ, Thirlby R, and Wolfe B, for The Longitudinal Assessment of Bariatric Surgery (LABS) Consortium: Perioperative safety in the longitudinal assessment of bariatric surgery. N Engl J Med 361: 445-454, 2009.

A WIN-Win Situation—The Weight-control Information Network's Getting on Track: Physical Activity and Healthy Eating for Men

The Weight-control Information Network (WIN), a national information service of the NIDDK, has developed a new brochure specifically for men entitled "Getting on Track: Physical Activity and Healthy Eating for Men." A significant number of U.S. adult men age 20 or older are overweight or obese based on body mass index (BMI), a measure of weight relative to height. Obese and overweight individuals have increased risk for developing serious diseases, including type 2 diabetes, cardiovascular disease, stroke, fatty liver disease, and certain types of cancer, compared to individuals of normal weight.

Moreover, there are gender-specific obesity-related health risks among obese men and women. Overweight men have greater overall health risks compared to overweight women, particularly during middle age. Although men typically have less body fat compared to women, they tend to store excess fat around their abdomen ("visceral fat"). Excess visceral fat substantially increases the risk of heart disease, metabolic syndrome, and type 2 diabetes.

To help men get fit and lose weight, WIN has published a new brochure, *Getting on Track: Physical Activity and Healthy Eating for Men*. In addition to providing information on BMI and risks associated with overweight and obesity, the brochure also includes information to help men "get on track" with healthy habits. These include tips to become more active (*e.g.*, walking daily, weight training, or taking the stairs rather than the elevator) and improve eating habits (*e.g.*, through increasing consumption of fruits and vegetables, and reducing portion size.) Additionally, the brochure explains how to set goals for safe and effective weight loss, offers tips on how these goals can be reached, and contains additional resources on weight loss for men.



This new brochure for men complements a variety of other publications that WIN has developed for men, women, and children. WIN was established in 1994 to provide the general public, health professionals, the media, and Congress with up-to-date, science-based information on obesity, weight control, physical activity, and related nutritional issues.

The copyright-free full text of *Getting on Track: Physical Activity and Healthy Eating for Men* is available online at: *www.win.niddk.nih.gov/publications/gettingontrack.htm*. Other WIN publications are available at: *http://win.niddk.nih.gov/*

STORY OF DISCOVERY

The Surprising Features of Fat

The body's adipose tissue—or fat—sustains life in times of famine, and it fuels physical activity and vital biological processes. Too much fat, however, is a recipe for metabolic disaster. For decades, scientists recognized that obesity is linked to type 2 diabetes and other diseases, but it wasn't clear why. After years of research, supported in large part by NIDDK, scientists now know that adipose tissue not only stores energy, it also sends signals to other parts of the body and the brain to regulate—or in the case of obesity, disrupt—metabolism. This research is informing the development of new therapeutic strategies.

Fuel for the Body: Fat Storage

Fat cells, also called adipocytes, harbor the body's energy reserves in droplets of lipids, or fat molecules. Previously, fat tissue was thought to be simply a storage area. However, there were hints that not all fat tissue is the same, and that the amount of body fat is regulated. In 1950, scientists described some unusually obese mice whose size seemed to result from an unidentified gene defect. Years later, NIDDK-supported researchers in the 1980s and early 1990s found that genetic factors influence people's propensity for excess weight gain as well as their body fat distribution—or where on the body an extra helping of dessert might end up. Studies also confirmed earlier observations that fat in certain locations confers heightened risk for type 2 diabetes. Researchers now refer to this particularly problematic fat as "visceral" fat—fat around the organs deep within the abdomen. In 1990, scientists in NIDDK's Division of Intramural Research discovered a protein called perilipin that surrounds the lipid storage droplets and controls fat storage. Researchers have since identified a number of proteins related to perilipin, and have found that the synthesis and breakdown of lipid droplets are highly regulated. Complementing research on fat cell function, researchers also identified a master regulator of fat cell formation. With NIDDK support, scientists in the early 1990s discovered that the protein PPAR-gamma directs certain types of immature, precursor cells to develop into fat cells. More recently, scientists used PPAR-gamma as a marker to trace the lineage of adipocytes and identify where, within fat tissue, fat-cell precursors reside. NIDDK-funded scientists also recently discovered an intriguing link between fat storage and the bacteria and other microbes that reside in the gut; certain gut microbes, and their collective genomes, seem to promote obesity.

Not Just a Storage Bin: Molecular Discoveries Linking Excess Fat to Metabolism and Disease

In 1994, NIDDK-supported scientists identified the *leptin* gene—a discovery that would ignite an explosion of research into the control of appetite and body weight, shine a spotlight on the role of adipose tissue in regulating metabolism, and change perceptions about obesity. A mutation in the *leptin* gene caused extreme obesity in the mice observed decades earlier. Subsequent research has shown that leptin protein, a hormone released by fat cells, travels to a key control center in the brain to update the status of the body's energy stores and reduce appetite. It also has effects elsewhere in the body. For people who lack leptin due to very rare genetic mutations, administration of the hormone effectively treats their extreme obesity, returning their weight to normal. Demonstrating a genetic and hormonal basis for excess body weight, this research also underscored that appetite control could no longer be viewed as solely a "will power" issue. Leptin treatment is not effective for people who have more common forms of obesity (which do not result from leptin deficiency), likely because other organs and tissues become resistant to leptin's actions. Researchers are now finding variants in other genes

and regions of the genome that may contribute to more common forms of obesity.

Since the discovery of leptin, NIDDK-supported researchers have elucidated a complex network of signaling molecules—several (like leptin) made by fat cells—that regulate appetite and energy expenditure. For example, adiponectin, secreted by fat cells, helps the body respond to the hormone insulin. In obesity, abnormally low levels of adiponectin are associated with insulin resistance, which is a risk factor for and hallmark of type 2 diabetes. By contrast, elevated levels of another factor, called RBP4, are associated with insulin resistance, type 2 diabetes, and cardiovascular disease risk. RBP4 is secreted by adipocytes, particularly those that comprise visceral fat. In other studies, researchers found a relatively high level of visceral fat in children who were obese and had pre-diabetes (high blood glucose conferring risk for diabetes). The children also had more fat in their muscles and liver, where it can cause metabolic problems. Pursuing research on a different fat-related condition, scientists in NIDDK's Division of Intramural Research, industry, and elsewhere have collaboratively shown that leptin can be used to treat metabolic problems associated with lipodystrophy, a rare group of disorders characterized by lack of fat in areas of the body where it should be, and abnormal fat accumulation in tissues such as liver and muscle.

Inflammation and Co-Conspirators Within Fat Tissue

Among the factors secreted by adipose tissue, several promote chronic inflammation, which has been linked to type 2 diabetes and cardiovascular disease risk. In 2003, NIDDK-funded researchers made the surprising discovery, first in mice and then in humans, that these factors are not all made by fat cells. Some are produced by cells of the immune system, macrophages, which infiltrate fat tissue. For example, macrophages within adipose tissue are the primary source of the pro-inflammatory factor TNF-alpha. Levels of another factor, resistin, are increased in obesity and contribute to insulin resistance. Originally identified as a fat cell-derived factor in mice, resistin interestingly is secreted by macrophages in humans.

Mind over Matter, and Matter over Mind

While the brain integrates an array of signals to control appetite and body weight, adipose tissue not only signals the brain to report on fat stores, but also exerts striking control over the brain's architecture and activity, as shown over the past several years by NIDDK-supported research. Scientists studying rodents discovered that leptin is involved in developing neural connections in the brain. In humans, researchers using advanced neuroimaging techniques found that the sight of food elicited different patterns of brain activity in obese people before and after weight loss. Leptin administration reversed many of these changes, and thus may potentially help people maintain weight loss.

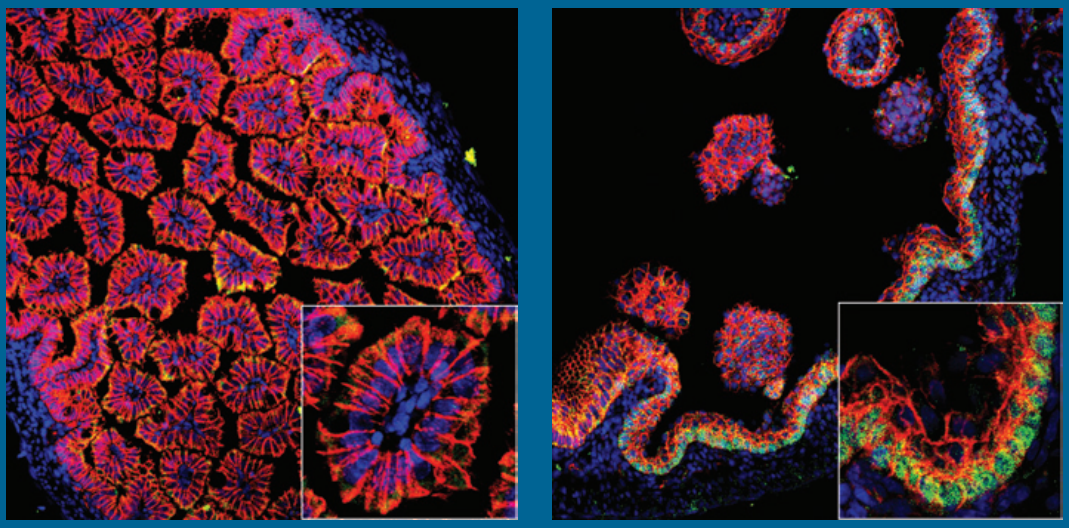
From Fuel to Furnace: "Brown" Fat Tissue

The adipocytes that store fat are called "white" fat cells. However, another type of fat, "brown" adipose tissue, burns fat to dissipate heat, and helps keep babies and small animals warm. While white adipose tissue stakes its territory in the body with a tenacity known all too well to those on a diet, brown fat tissue was thought to disappear after infancy in humans. In 2009, however, NIDDK-supported scientists and other research teams discovered that brown fat is present in adult humans and appears metabolically active with exposure to cooler temperatures. They also found that people who are overweight or obese have less active brown fat. In studies in rodents, NIDDK-funded scientists are illuminating the molecular pathways that trigger brown adipocyte development. These experiments revealed that at least some brown fat can arise from the same precursor cells as another energy-burning tissue—muscle. Other brown fat cells may share a lineage with white adipose cells. These findings may lead to

a novel strategy for treating obesity: generating more brown fat cells to burn excess calories.

Future Directions

These discoveries about the multifaceted nature of fat tissue have revolutionized thinking about obesity and elucidated potential targets for future therapeutic development. As scientists advance technology for studying adipose tissue and measuring its effects in animal models and humans, new insights will emerge, along with new strategies to reduce excess fat and prevent the serious diseases associated with obesity.



These sections of small intestine from normal mice (left panel) and mutant, *Cdx2*-deficient mice (right panel) show overall disorganization in the mutant mouse intestine, as well as aberrant production of proteins typically found in the upper gastrointestinal tract (esophagus). Smaller inset boxes show magnified images. As described in this chapter, researchers have identified Cdx2 as an essential factor controlling formation of normal small intestine, with implications for some intestinal conditions that affect humans as well.

Images provided by Dr. Klaus H. Kaestner and reprinted from Developmental Cell, 16, Gao N, White P, and Kaestner KH, Establishment of intestinal identity and epithelial-mesenchymal signaling by Cdx2, 588-599, Copyright 2009, with permission from Elsevier.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of doctor visits, hospitalization, and disability in the U.S. each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. In 2004, over 35 percent of all emergency and outpatient hospital visits—some 100 million—were associated with a diagnosis of a digestive disease.[1] With some being very common and others quite rare, digestive diseases, collectively, exact a significant toll on public health in terms of their effects on quality of life, years lost due to death, and costs associated with hospitalization and pharmaceutical and surgical interventions. To reduce the public health burden associated with digestive diseases, NIDDK-supported scientists are vigorously pursuing research to understand how widespread these diseases are across the U.S., to identify the causes of these diseases and how they progress, and to test new interventions for prevention and treatment of these costly diseases, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. IBD often strikes early in life, with a peak age of onset in adolescence or young adulthood. To address this condition, surgery may be required, including removal of the affected region of the intestine. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. The continued discovery of predisposing genetic variations and potential autoimmune and microbial influences will help catalyze the design of novel therapeutic strategies.

Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer. Screening programs for colorectal cancer are aimed at reducing mortality through early detection, particularly in those individuals at higher risk.

Intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus transform into an intestinal-type cell, is associated with a heightened risk of esophageal cancer, the most rapidly rising cancer in the U.S. Gastroparesis is another functional bowel disorder that is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. A common cause of gastroparesis is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden, particularly in the elderly.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat,

[1] *Everhart JE, editor. The burden of digestive diseases in the U.S. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: U.S. Government Printing Office, 2008; NIH Publication No. 09-6443.*

barley, and rye—and results in damage to the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, growth failure. The only current treatment for celiac disease is maintenance of a strict gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. The microbes can affect intestinal health in some surprising ways, depending on their interactions with each other, with host cells, and with nutrients ingested by their host. Scientists are gaining insights into the ways these GI microorganisms influence the development and function of the digestive tract, as well as other systems throughout the body such as those with immune and metabolic functions.

The liver is an organ within the digestive system that performs many centralized functions in the body, including metabolism and distribution of nutrients such as fats. When the liver is functionally compromised by disease, this can have serious adverse impacts on health and can sometimes lead to complete liver failure. Some liver diseases primarily affect children—such as biliary atresia, a progressive inflammatory liver disease—while others more commonly affect adults—such as a form of nonalcoholic fatty liver disease known as nonalcoholic steatohepatitis. Some are caused by viral infection such as hepatitis B and C, or by genetic mutations such as alpha-1-antitrypsin deficiency, while others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many of these forms of liver disease, such as chronic hepatitis C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is of critical importance to identify liver disease, preserve liver function in people with liver disease, and develop new treatment options, including transplants performed with liver tissue from living donors.

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous serious diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, and environmental factors that influence appetite, metabolism, and energy storage, they are identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

GENETICS OF INFLAMMATORY BOWEL DISEASES

New Genetic Risk Factors Identified for Ulcerative Colitis: An international research group, including investigators from the NIDDK's IBD Genetics Consortium, has identified new genetic risk factors associated with ulcerative colitis (UC) through a genome-wide association study of over 1,000 people with UC compared to other individuals without the disease.

UC causes inflammation in the tissue lining the colon and rectum, which may result from abnormal immune responses within the intestines. Although UC shares some features with the other major form of IBD—Crohn's disease—other characteristics are distinct. Genome-wide association studies in recent years have identified genetic factors that contribute to each of these forms of IBD. These studies have been particularly fruitful in terms of uncovering genetic regions associated with Crohn's disease. In the current study, researchers intensified their efforts to identify additional genetic factors that increase susceptibility to UC.

To expand knowledge of genetic contributors to UC, researchers performed a genome-wide association study using DNA collected from individuals with or without UC who shared a similar ancestry, in order to minimize other genetic differences. With this method, they were able to identify chromosomal regions, as well as genes within some of those regions, that are associated with an increased risk of developing UC. Two chromosomal regions were linked for the first time to UC risk. Several genes located within or near these regions play a role in immune function and inflammation, and may contribute to disease susceptibility by altering these processes. Additional genetic factors previously implicated in UC and Crohn's disease, including the immune system gene *IL-23R*, were also confirmed in this analysis. However, many genetic factors that had proven important for Crohn's disease risk were not associated with susceptibility to UC, suggesting that the two forms of IBD have overlapping but unique genetic profiles.

The identification of genetic regions associated with increased susceptibility to UC has the potential to inform understanding of disease processes unique to this form of IBD. Additionally, this knowledge can provide targets for developing new, more personalized therapeutic and preemptive approaches to controlling this disease.

Silverberg MS, Cho JH, Rioux JD, McGovern DPB, Wu J, Annese V, Achkar JP, Goyette P, Scott R, Xu W, Barmada MM, Klei L, Daly MJ, Abraham C, Bayless TM, Bossa F, Griffiths AM, Ippoliti AF, Lahaie RG, Latiano A, Paré P, Proctor DD, Regueiro MD, Steinhart AH, Targan SR, Schumm LP, Kistner EO, Lee AT, Gregersen PK, Rotter JI, Brant SR, Taylor KD, Roeder K, and Duerr RH: Ulcerative colitis–risk loci on chromosomes 1p36 and 12q15 found by genome-wide association study. Nat Genet 41: 216-220, 2009.

SCREENING FOR COLON CANCER

Studies of Polyp Size Inform New Recommendations: Scientists have provided recommendations for managing patients with colon polyps based on new evidence of the relationship of polyp size to the risk of colon cancer. These recommendations have been incorporated into new screening guidelines to better predict and prevent colon cancer.

Imaging methods being used for colon cancer screening—such as computer tomographic colonography (CTC)—are preferred by some patients and clinicians to colonoscopy, but they present new challenges. Although these methods can detect polyps and assess their size, unlike colonoscopy, they do not provide a way to detect tissue abnormalities or remove cancerous or pre-cancerous polyps (polypectomy). Clinicians must decide which patients with polyps detected by these methods should receive follow-up colonoscopy and polypectomy. Information on how polyp size relates to colon cancer risk would help to inform the best screening and management practices.

To gain insight into how polyp size indicates whether a polyp is likely to become cancerous, scientists analyzed reports on screening colonoscopies of asymptomatic patients. Polyps that were identified during the colonoscopies were removed and analyzed for tissue abnormalities. Patients with polyps were grouped by the size of their largest polyp, and the polyps were categorized according to their tissue characteristics. Analyses of polyp size and abnormalities supported current screening guidelines that patients with large polyps should be offered colonoscopy and polypectomy based on the high risk that the polyp was pre-cancerous. The study results also agreed with current guidelines stating that small polyps present very low risk; therefore, most of these patients could be followed safely with periodic imaging rather than polyp removal, although additional research was recommended. Notably, the study analyses indicated that intermediate-size polyps present a substantial risk. Based on these results, it was recommended that patients with mid-size polyps should be offered colonoscopies and polypectomies. Also, the scientists suggested that another study be conducted to assess the cost-effectiveness of image-based screening for colon cancer if a significant number of patients will require follow-up colonoscopies under these new recommendations.

Current colon cancer screening and management guidelines, written by several professional societies, have been updated to reflect the recommendations of this study regarding use of colonoscopy and polypectomy depending on polyp size. For patients with polyps, this more informed management strategy should improve their care and chances of avoiding colon cancer.

Lieberman D, Moravec M, Holub J, Michaels L, and Eisen G: Polyp size and advanced histology in patients undergoing colonoscopy screening: Implications for CT colonography. Gastroenterology 135: 1100-1105, 2008.

INTESTINAL DEVELOPMENT, HOST DEFENSE, AND WOUND REPAIR

Master Regulator of Intestinal Development Identified: Scientists have found that a single gene plays an essential role in controlling normal intestinal development. *In utero*, the GI tract develops from the foregut, which becomes the esophagus, stomach, and upper part of the small intestine; the midgut, which gives rise to the small intestine; and the hindgut, which forms the cecum and colon. The formation of the intestine is directed by a complex network of precisely timed signals passing between and within cells of these developing tissues, including layers of cells that will form the lining of the intestine (epithelial cells) and cells that will form intestinal smooth muscle and other intestinal tissues (mesenchymal cells). Some of these signals are so essential for life that, when mutated, the resulting deficiency causes death *in utero*. For example, mutation of a mouse gene important to intestinal development, called *Cdx2*, is lethal for mice at the embryonic stage. Humans also carry a form of this gene, referred to as *CDX2*.

To investigate the role that the *Cdx2* gene plays specifically in development of the intestine, scientists created a mouse model in which the *Cdx2* gene is only mutated in developing intestinal cells; with this restricted *Cdx2* deficiency, the developing mice do not die *in utero* and can thus be studied. When the scientists compared this mutant mouse model with normal mice, they observed some dramatic and surprising changes in the development of the intestine. For example, the colon in the mutant mouse failed to develop correctly, mimicking a disorder in humans known as colonic atresia. Also, epithelial cells on the inner layer of the intestine did not differentiate to form the correct cell types and structures, such as villi with brush border membranes needed for nutrient absorption. Instead, the mutant epithelial cells more closely resembled cell types found in the upper GI tract, specifically the esophagus, rather than those found in a normal intestine. Analyses of the "transcriptome"—the collection of genes turned on (expressed)—in the small intestine confirmed that the *Cdx2*-deficient small intestine had converted to a more esophageal type by turning on genes typically seen in the esophagus but not the intestine. As the intestine develops, *Cdx2* production normally becomes restricted to epithelial cells. However, the researchers showed that cells of the other tissue layers within the intestine were also altered to be more esophagus-like, suggesting that *Cdx2* deficiency-related epithelial modifications subsequently caused changes in adjacent tissue layers, converting them to an esophageal program as well.

These experiments demonstrate the importance of *Cdx2* to proper intestinal development by characterizing the repercussions of its removal, including abnormal colon formation and a switch to an upper GI tract phenotype. Based on these findings, *Cdx2* is now recognized as playing a critical role in programming tissues along the length of the GI tract to have specific cell types and features to suit their different functional needs. Beyond informing our understanding of intestinal development, these studies have implications for human conditions in which intestinal programming is altered, such as colonic atresia.

Gao N, White P, and Kaestner KH: Establishment of intestinal identity and epithelial-mesenchymal signaling by Cdx2. *Dev Cell 16: 588-599, 2009.*

Specialized Cells in the Intestine Prevent Bacteria from Invading Other Tissues: Scientists have recently conducted studies in mice that provide new insights into how specific cells lining the intestine detect and respond to resident bacteria and maintain a mutually beneficial relationship with the intestinal microbial community. The intestines of humans and other animals provide a home to billions of bacteria. This microbial community and our own cells have developed a symbiotic relationship in which bacteria provide essential metabolic functions in exchange for a nutrient-rich environment. It is important, however, that microbes in the gut do not spread to other tissues, where they could cause damage, and even death. Resident bacteria are confined to the gut by a layer of cells that make up the intestinal lining. A specialized type of these cells, known as Paneth cells, protects the host against breaches in the intestinal barrier by

initiating an antimicrobial response, which could target either normal gut bacteria or invading pathogens. Given the protective importance of this response, scientists are interested in understanding how Paneth cells detect bacteria to initiate the antimicrobial program that helps maintain symbiotic balance in the gut.

To understand the role of Paneth cells in detecting and responding to intestinal bacteria, scientists carried out experiments in mice lacking a key cellular signaling protein, called MyD88. In these experiments, they found that mice lacking MyD88 in all of their cells were unable to initiate an antimicrobial response. However, reintroduction of MyD88 specifically into Paneth cells—but not other types of cells—of these mice was sufficient to stimulate the antimicrobial response to gut bacteria. This result demonstrated that Paneth cells directly detect bacteria to stimulate MyD88 activation, rather than relying on signals from MyD88 activation in neighboring cells. The scientists went on to show that mice lacking MyD88 also had increased numbers of bacteria in tissues outside of the intestine compared to normal mice. By looking further at mice that do not have Paneth cells, they showed that MyD88 signaling in Paneth cells is essential for restricting the movement of gut bacteria across the intestinal lining into host tissues.

This study showed that Paneth cells limit the penetration of bacteria across the intestinal barrier, perhaps by regulating how the bacteria interact with cells of the intestinal lining. Since elevated levels of bacteria associated with the intestinal lining have been observed in patients with IBD, these results may have important implications for understanding the pathology of the disease.

Vaishnava S, Behrendt CL, Ismail AS, Eckmann L, and Hooper LV: Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc Natl Acad Sci USA 105: 20858-20863, 2008.

Immune Cell Protein Regulates Wound Repair in the Colon: By studying how injuries to the colon lining are repaired in normal and genetically-engineered mice, scientists have unveiled the role of an immune cell protein, called Trem2, in mediating efficient wound repair in the colon. Following injury to a tissue, the body orchestrates an inflammatory response to stave off potential infections and repair the damaged tissue. This process is particularly important in the small and large intestines (including the colon), where a lining of cells, called epithelial cells, provides the barrier between the inside (lumen) of the intestines and the deeper tissue layers, as well as surrounding tissues. Since the lumen of the intestines is home to trillions of bacteria, some of which play essential roles in intestinal processes such as normal metabolism while others are potential pathogens, the integrity of the intestinal lining must be maintained to prevent the spread of harmful microbes within the gut and to other parts of the body. Therefore, any breaches in the intestinal barrier due to injury must be efficiently repaired to prevent localized and systemic infection.

To study the cellular and molecular basis for intestinal wound repair, scientists adapted a biopsy technique widely used on skin to generate uniform wounds in the colon of normal and genetically-engineered mice. By analyzing the regeneration process following biopsy injury, the scientists found that wound healing occurred in two phases. In the first phase (1-2 days after injury), a single layer of cells formed over the wound area by contraction of the surrounding tissue where stem cells were located. Subsequently, there was an increase in epithelial cell growth and division adjacent to the wound area and an increase in the number of immune cells in the wound bed during the second phase (2-4 days after injury). More importantly, the scientists found that the two phases of wound healing were associated with the presence of different types of immune cells and the chemicals they release, known as cytokines, in and around the wound bed. In addition, they showed that the switch from phase 1 to phase 2 of wound healing is triggered by a protein known as Trem2, which is located on the surface of immune cells called macrophages. Genetically-engineered mice that lacked Trem2 on their macrophages exhibited a sustained increase in phase 1 signals and a reduction in phase 2 signals that resulted in slow and incomplete wound healing. Thus, cellular signaling through Trem2 is important for promoting efficient healing of colonic wounds.

With a better understanding of the cellular and molecular components of wound healing in the colon, the researchers plan further studies to support their

model in which Trem2 subdues pro-inflammatory signals associated with phase 1, and boosts phase 2 signals to promote efficient wound healing. Understanding the molecular components of this process may provide targets for patients with defects in barrier function or immune cell activation that result in impaired colon repair following injury.

Seno H, Miyoshi H, Brown SL, Geske MJ, Colonna M, and Stappenbeck TS: Efficient colonic mucosal wound repair requires Trem2 signaling. Proc Natl Acad Sci USA 106: 256-261, 2009.

NEW THERAPEUTIC STRATEGY TO TREAT BARRETT'S ESOPHAGUS

Treating Pre-Cancerous Barrett's Esophagus: Barrett's esophagus has been treated successfully in a clinical trial using a nonsurgical procedure known as radiofrequency ablation (RFA). Barrett's esophagus is a condition that can result from gastroesophageal reflux disease. This happens when stomach acids flow back up into the esophagus, causing the cells lining the esophagus to transform into a type of cell that normally lines the intestine. In some patients with Barrett's esophagus, these cells continue to change, first becoming pre-cancerous and then developing into a deadly form of cancer known as esophageal adenocarcinoma. Currently, the only treatment for esophageal cancer is surgical removal of the esophagus. It has not been clear how best to treat Barrett's esophagus in which there are abnormal, pre-cancerous cells.

In a multi-center clinical trial supported by the NIDDK, patients with pre-cancerous Barrett's esophagus were divided into two groups and treated with either RFA or a "sham" procedure. RFA is an outpatient procedure using a catheter with a small balloon attached to its end that is inserted into the esophagus. When it is in place, the inflated balloon radiates heat, discretely destroying only the adjacent layers of abnormal cells lining the esophagus of the patient with pre-cancerous Barrett's esophagus. RFA is also less invasive than surgery, although this study did not assess the relative efficacy of surgery. The "sham" procedure given to the control group consisted of insertion of the catheter without destruction of abnormal tissue. Patients were given up to four RFA or "sham" sessions and then evaluated at the end of the year-long study period. When the RFA-treated patients were evaluated, most no longer had pre-cancerous esophageal cells. Only a very small number of RFA-treated patients had progression of their disease, and although some patients had reverted to early stage (not pre-cancerous) Barrett's esophagus, the majority of RFA-treated patients now had normal esophageal tissue. In contrast, only a small number of patients receiving the "sham" treatment improved, and a much larger number than the RFA group had disease progression. This study presents important findings by demonstrating that the RFA outpatient procedure is highly effective in treating Barrett's esophagus and reducing the risk that this disease will develop into a deadly form of esophageal cancer.

Shaheen NJ, Sharma P, Overholt BF, Wolfsen HC, Sampliner RE, Wang KK, Galanko JA, Bronner MP, Goldblum JR, Bennett AE, Jobe BA, Eisen GM, Fennerty MB, Hunter JG, Fleischer DE, Sharma VK, Hawes RH, Hoffman BJ, Rothstein RI, Gordon SR, Mashimo H, Chang KJ, Muthusamy VR, Edmundowicz SA, Spechler SJ, Siddiqui AA, Souza RF, Infantolino A, Falk GW, Kimmey MB, Madanick RD, Chak A, and Lightdale CJ: Radiofrequency ablation in Barrett's esophagus with dysplasia. N Engl J Med 360: 2277-2288, 2009.

CELIAC DISEASE RESEARCH

The Growing Burden of Undiagnosed Celiac Disease: Scientists have found evidence that the number of people with undiagnosed celiac disease has grown over the past 50 years, placing more individuals at a greater risk of premature death. Celiac disease is an autoimmune disease caused by intolerance of the gluten proteins found in many grains, which can be treated very effectively with a gluten-free diet. However, this disease often goes undiagnosed and untreated, often causing severe health problems.

To determine the consequences of undiagnosed celiac disease and to clarify whether its prevalence has changed over the past several decades, scientists used blood samples from three different groups of individuals to test for two antibodies associated with celiac disease. Samples from a group of U.S. Air Force personnel had been taken and stored frozen 45 years earlier, and were shown to be well-preserved. More recent samples were obtained from an older group of men with birth dates similar to those of the Air Force

personnel and from a younger group who were similar in age to the Air Force personnel when they were sampled. Individuals with both of the celiac-specific antibodies were considered to have undiagnosed celiac disease. When scientists compared the death rates for Air Force personnel who had undiagnosed celiac disease with those who did not, they discovered that death rates from all causes were nearly four-fold higher for those with undiagnosed celiac disease. Analyses comparing samples from the Air Force group with those from the older and younger groups tested revealed that the prevalence of undiagnosed celiac disease has increased more than four-fold over the past 50 years.

This study presents a dramatic picture of the rapidly increasing rate of undiagnosed celiac disease and its impact on the U.S. population. Its findings illustrate the importance of alerting clinicians and the public to this multi-symptom, often-overlooked disease so that diagnosis can be made before severe health problems develop. Toward this end, the NIDDK's Celiac Disease Awareness Campaign informs health care personnel and the public about celiac disease, its treatment, and its consequences. The Campaign can be accessed at *www.celiac.nih.gov/*

Rubio-Tapia A, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, Brantner TL, Kim WR, Phelps TK, Lahr BD, Zinsmeister AR, Melton LJ 3rd, and Murray JA: Increased prevalence and mortality in undiagnosed celiac disease. Gastroenterology 137: 88-93, 2009.

Molecular Basis for Immune Reactivity to Dietary Gluten in Celiac Disease: Researchers supported by NIDDK have uncovered how a variant of an immune system molecule associated with celiac disease contributes to increased immune reactivity towards dietary gluten. Celiac disease is an autoimmune disease in which an aberrant immune response to the gluten protein found in dietary grains, such as wheat, barley, and rye, results in chronic inflammation and tissue damage in the intestine. The increased gluten sensitivity in a subset of celiac patients is strongly associated with HLA-DQ8, a variant of the HLA protein molecule that is found on the surface of cells. Normally, HLA molecules present potentially harmful substances to the immune system. In patients with celiac disease, however, HLA-DQ8 presents fragments of gluten proteins to immune system cells, called T cells, via "receptors" on these cells, known as T-cell receptors (TCRs). This interaction initiates an aberrant immune response against the gluten protein. However, it is not fully understood how this particular version of the HLA molecule interacts with gluten and generates an immune response.

Previous studies have shown that the HLA-DQ8 protein has a sequence variation compared to other HLA molecules, which confers a preference for detecting protein fragments classified chemically as having a negative charge. Although gluten fragments do not inherently contain negative charges, they can be modified by cellular enzymes to contain negative charges. This modified gluten fragment is thought to elicit the HLA-DQ8-mediated immune response. In the present study, scientists showed that while the modified, negatively charged gluten fragment has a functional advantage in eliciting an immune response, HLA-DQ8 can detect and generate an immune response to the unmodified, native fragment as well. They found that the ability to incite an immune response to both modified and unmodified gluten fragments requires signaling through different sets of TCRs. For the negatively charged fragment, an immune response is generated by signaling through a broad repertoire of TCRs that possibly recognize the fragment in slightly different ways. In contrast, a very limited set of TCRs is used to detect the unmodified, native gluten fragment. Analysis of TCR sequences showed that this limited set of TCRs contains a unique negative charge that is required for recognizing the unmodified, native gluten fragment. By introducing mutations that remove this negative charge from the TCR, the researchers found that the immune response to unmodified gluten fragments can be abolished. In addition, they found that sensitivity to the native, unmodified gluten fragment is associated with TCRs that all contain the conserved negative charge in cell lines derived from patients with celiac disease.

The results of this study reveal a new mechanism by which the immune cell molecule HLA-DQ8 detects gluten fragments to elicit an immune response in celiac disease. The researchers propose that the initial response to the native fragment followed by a much broader response to the modified fragment contribute to the amplified gluten sensitivity in celiac disease,

paving the way for further investigations into how this response contributes to disease onset and progression. Future research along these lines could also determine whether HLA-DQ8 contributes through a similar mechanism to type 1 diabetes, with which it has also been associated.

Hovhannisyan Z, Weiss A, Martin A, Wiesner M, Tollefsen S, Yoshida K, Ciszewski C, Curran SA, Murray JA, David CS, Sollid LM, Koning F, Teyton L, and Jabri B: The role of HLA-DQ8 beta57 polymorphism in the anti-gluten T-cell response in coeliac disease. Nature 456: 534-538, 2008.

RESEARCH ON HEPATITIS C

Long-Term Treatment with Common Hepatitis C Therapy Does Not Prevent Liver Disease Progression: Results of a large, multi-center clinical trial provide strong evidence that long-term therapy with a standard antiviral drug used to treat chronic hepatitis C is ineffective at preventing disease progression in patients who did not initially respond to a shorter course of antiviral treatment.

Chronic hepatitis C is the major cause of cirrhosis and liver cancer in the U.S. The standard treatment for chronic hepatitis C—a combination of the antiviral drugs peginterferon and ribavirin given for 24 to 48 weeks—is associated with some side effects and is not effective for half of patients, for whom additional treatment options are currently limited. In those for whom standard treatment is ineffective, researchers investigated whether longer-term treatment with peginterferon might prompt a response in terms of reduced progression to cirrhosis and a form of liver cancer known as hepatocellular carcinoma.

The Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) trial was designed to test whether long-term antiviral therapy can prevent liver disease progression in people with advanced hepatitis C who did not respond previously to standard, short-term therapy. These individuals were given either peginterferon or no treatment for 3.5 years. Liver biopsies were collected to assess progression of disease to endpoints such as cirrhosis and hepatocellular carcinoma. Although levels of key liver enzymes and levels of hepatitis C virus were significantly reduced in patients receiving peginterferon compared to those receiving no treatment, the treatment did not reduce progression of liver disease. This result indicates that there is no benefit to continuing peginterferon therapy long-term in those with chronic hepatitis C who do not respond to a standard course of antiviral therapy.

The results of this trial can help spare patients from ineffective long-term treatment and its side effects. The trial also provides renewed incentive to find new therapies for chronic hepatitis C, which may include some experimental agents currently in early stages of development.

Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, Lok AS, Bonkovsky HL, Morgan TR, Ghany MG, Morishima C, Snow KK, Dienstag JL, for the HALT-C Trial Investigators: Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med 359: 2429-2441, 2008.

Scientists Discover Genetic Factors in Liver Cells Needed for Hepatitis C Virus Infection: A team of scientists working in the NIDDK's intramural Liver Diseases Branch has applied genomic technology to identify genetic factors in liver cells that are used by the hepatitis C virus (HCV) for successful infection. Hepatitis C is one of the major causes of chronic liver disease in the U.S., and is a common cause of liver cancer. HCV invades liver cells of infected individuals, co-opting the cellular machinery to produce more copies of the virus. A wide spectrum of response to HCV infection is seen across individuals, in that some develop severe chronic liver disease while others spontaneously purge the virus. Available therapy for hepatitis C is effective in only some patients and can cause significant side effects. Some patients with hepatitis C are co-infected with the human immunodeficiency virus (HIV), further complicating their care.

Building on these observations and recent technological achievements, such as the development of a cell culture system for studying HCV, and genome-wide screening and RNA interference technology, these researchers

screened HCV-infected liver cells to identify genetic factors that are required for successful HCV infection. Using molecules called "small interfering RNAs" to selectively inhibit production of particular genetic factors within HCV-infected human liver cells, the scientists screened the effect of silencing different genes throughout the genome on viral production. The screen identified 30 genetic factors in the liver cells as being important for viral production—factors that were previously implicated in HCV infection—demonstrating the screen's validity to correctly pick out meaningful factors. It also identified several previously unappreciated genetic factors in liver cells on which HCV depends for infection. The researchers compared their findings of genetic factors in liver cells needed for HCV infection to viral factors identified by an HCV infection mapping project to visualize important interactions between host and viral proteins. Comparison to other viruses related to HCV, such as the West Nile Virus, also revealed several host factors and mapped host-viral interactions used by both viruses to cause infection. This study also compared genetic factors in liver cells found to be important for HCV infection with those for HIV infection, revealing 10 liver cell factors in common that are used by both viruses.

This study provides a wealth of information on genetic factors in liver cells that enable HCV infection. Pursuit of these genetic leads has the potential to identify targets for future therapies that are personalized to be more effective in individuals whose HCV infection is unresponsive to current treatment. This work may also benefit those who are co-infected with both HCV and HIV by forming the basis for therapies that target host genetic factors on which both viruses depend.

Li Q, Brass AL, Ng A, Hu Z, Xavier RJ, Liang TJ, and Elledge SJ: A genome-wide genetic screen for host factors required for hepatitis C virus propagation. Proc Natl Acad Sci USA. 106: 16410-16415, 2009.

GENETICS OF PRIMARY BILIARY CIRRHOSIS

Genetic Variants Linked to Autoimmune Disease Targeting Bile Ducts: Researchers have identified several genetic variants that are commonly found in patients with primary biliary cirrhosis. In this chronic autoimmune disease, the bile ducts in the liver become inflamed and damaged and, ultimately, disappear. When this happens, bile—a liquid produced in the liver and released through bile ducts to aid digestion of dietary fats in the intestine—builds up in the liver and leads to liver damage, cirrhosis, and end-stage liver disease. The initial inflammation and damage to bile ducts is believed to result from an autoimmune response in which the body's immune system inadvertently attacks and destroys specific cells lining the bile ducts. While scientists know the molecular factor that elicits the immune response, it is not fully understood why this molecule is inappropriately targeted in the first place. Like other autoimmune diseases, scientists think that the underlying cause of primary biliary cirrhosis has a strong genetic component. However, no genetic factors had been conclusively identified that point to a cause for primary biliary cirrhosis.

To facilitate the identification of possible genetic links to primary biliary cirrhosis, NIDDK-supported scientists recently carried out a genome-wide association analysis of DNA samples from patients with the disease compared to healthy controls. In this type of study, scientists scan the genomes of thousands of individuals and look for genetic variants that are more likely to be associated with a particular trait or condition such as, in this case, primary biliary cirrhosis. By scanning the genomes of over 500 patients with primary biliary cirrhosis and over 1,500 healthy individuals, this analysis identified several genetic variants in three specific regions—or loci—of the genome that are more frequently found in the patient group (and not the healthy control group). One of these regions, called the *HLA* locus, codes for proteins that are important for presenting molecules to the immune system to initiate the immune response; variants in genes coding for particular HLA proteins are often associated with other autoimmune diseases. The other two regions lie within the *IL12A* and *IL12RB2* genetic loci, which code for a pair of proteins—interleukin-12 (IL-12) and the IL-12 receptor—that are critical for cellular communications to propagate the inflammatory immune response. By conclusively associating genetic variants in these loci with primary biliary cirrhosis, this study sheds light on the "immunogenetic" basis of this disease and

points to the IL-12 signaling pathway as a potential therapeutic target for treating patients.

Hirschfield GM, Liu X, Xu C, Lu Y, Xie G, Lu Y, Gu X, Walker EJ, Jing K, Juran BD, Mason AL, Myers RP, Peltekian KM, Ghent CN, Coltescu C, Atkinson EJ, Heathcote EJ, Lazaridis KN, Amos CI, and Siminovitch KA: Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. N Engl J Med 360: 2544-2555, 2009.

Childhood Liver Disease Research and Education Network

Although liver diseases are considered rare during childhood, those that do occur tend to be severe and progressive, often occur for unknown reasons, and have limited effective medical therapies. In June 2009, in an effort to improve the understanding of pediatric liver disease and to improve the care of children with these conditions, NIDDK established the Childhood Liver Disease Research and Education Network (ChiLDREN). ChiLDREN, which is a collaborative team of doctors, scientists, medical facilities, and patient support organizations, brings together ongoing clinical research efforts of the Biliary Atresia Research Consortium (BARC) and the Cholestatic Liver Disease Consortium (CLiC) under one cohesive network. The consolidation of BARC and CLiC resources into one network will better enable clinicians to conduct clinical trials, facilitate the discovery of underlying causes of disease, and lead to the development of new diagnostic and treatment options for children with liver disease.

BARC was initially funded by NIDDK in 2002 to further understand the causes of and develop treatments for biliary atresia, the most common, severe liver disease in children. In children with this condition, the abnormal formation or absence of bile ducts results in the blockage of bile flow from the liver (cholestasis) and liver injury. Although surgical procedures are available to treat children with biliary atresia, these procedures are usually not curative and most children will eventually require a liver transplant; biliary atresia is the most common cause for liver transplants in children. The causes of this disease are unknown.

To better understand the causes of biliary atresia and develop improved treatments, BARC is currently carrying out several clinical studies. In two of these studies, clinicians are collecting clinical information and biological samples (tissues and body fluids) from infants and children with biliary atresia. These types of prospective, observational studies will allow researchers to identify potential causative factors that lead to disease onset and follow the natural history of disease progression. In a third clinical trial, researchers are evaluating the use of corticosteroid therapy following the standard surgical procedure—known as the Kasai procedure—for treating infants with biliary atresia, with the hope of improving the long-term benefits of surgery and reducing the need for liver transplant in infants with biliary atresia.

While biliary atresia does not appear to be an inherited condition, several other cholestatic liver diseases have an inherited genetic basis. Initially established in 2004 as part of the NIH Rare Disease Clinical Research Network and now part of the NIDDK-funded ChiLDREN, CLiC is studying five distinct genetic causes of inherited pediatric cholestatic liver disease: Alagille syndrome, alpha-1-antitrypsin deficiency, progressive familial intrahepatic cholestasis, bile acid synthesis defects, and mitochondrial hepatopathies. The major goal of CLiC is to follow the natural history and progression of these diseases. In an ongoing clinical study, called Evaluating the Genetic Causes of Progression of Cholestatic Liver Diseases, researchers are collecting clinical information, family history, and biological samples from children with cholestatic liver diseases, so as to develop a better understanding of the causes and effects of these diseases. Progress in identifying the molecular and biochemical causes will offer researchers new opportunities for developing therapeutic interventions.

In a new area of research, CLiC has recently expanded to include the study of liver disease in people with cystic fibrosis. Up to 30 percent of patients with cystic fibrosis will develop liver disease, which can lead to cirrhosis and end-stage liver disease. To better understand the development of liver disease in patients with cystic fibrosis, CLiC has initiated studies to evaluate the natural history of cystic fibrosis liver disease (CFLD) and to identify predictors of developing liver disease in patients with cystic fibrosis and predictors of outcome in children with CFLD. In June 2009, NIDDK, in collaboration with NIH Office of Rare Diseases and the Cystic Fibrosis Foundation, convened the "Cystic Fibrosis Liver Disease Clinical Research Workshop" to discuss the prevalence, cause, diagnosis, and treatment of CFLD. An assessment of the state-of-the-science in CFLD research highlighted

the need for continued development of non-invasive methods for diagnosing and monitoring the progression of liver disease in patients with cystic fibrosis.

NIDDK-supported research conducted by ChiLDREN will lead to a better understanding of the causes and progression of cholestatic liver diseases in children. Advances from ongoing basic, clinical, and translational research will lay the foundation for the discovery of new diagnostic markers and innovative therapies to help improve the care of children with these diseases.

STORY OF DISCOVERY

Bacteria in the Intestines Are Linked to Nutrient Metabolism and Obesity

The epidemic level of obesity in the U.S. presents an enormous impact on public health, as obesity is strongly associated with many serious diseases, such as type 2 diabetes, heart disease, and certain types of cancer. Thus, the NIH has continued to pursue a multi-dimensional research agenda on the molecular, physiological, behavioral, and environmental factors that contribute to obesity, along with research on prevention strategies and treatment of obesity. Among the many advances emerging from recent obesity research, one surprising and intriguing finding—pioneered by NIDDK-supported scientists—is that the bacteria that naturally reside in the digestive system may influence weight gain.

The human intestine is host to an enormous ecosystem of microorganisms. In fact, with a population of nearly 100 trillion, bacterial cells in the intestines outnumber human cells by almost ten to one. In exchange for a nutrient-rich environment, this bacterial community provides essential metabolic functions that humans have not developed on their own. The intimate, symbiotic relationship between the bacterial community and human physiology has even led some researchers to consider humans not as a single organism, but instead as a "supraorganism" composed of both human genes and bacterial genes. Understanding the composition of the bacterial genes and how they interact with human genes to contribute to normal health and disease has become a focal point of several NIH research initiatives.

While the presence of bacteria in the intestines and their impact on human health has been appreciated for over a century, it was only recently that researchers made a connection between the microorganisms and obesity. In 2002, Dr. Jeffrey Gordon, then a member of NIDDK's National Advisory Council, presented to the Council his vision of the future of digestive diseases research as encompassing the microbial world within us, and proposed that gut microbes might be associated with obesity. In 2004, Dr. Gordon and his research team at Washington University School of Medicine, in St. Louis, first published a study showing that the community of bacteria in the large intestine is an important "environmental factor" that affects how energy is extracted from the diet and stored as fat. By "transplanting" intestinal bacteria from normal mice into germ-free mice that had never been exposed to bacteria, Dr. Gordon's team showed that the bacterial community can turn on and off host genes that are important for the production and storage of fat. This led the researchers to propose that individuals who are obese may have intestinal bacteria that are more efficient at extracting and storing energy—calories—from the diet compared to the bacterial population in individuals who are not obese.

Dr. Gordon and his colleagues tested this hypothesis in two landmark follow-up studies. In the first study, they analyzed bacterial DNA sequences to compare the types of bacteria found in the large intestine of normal mice and mice that were genetically engineered to be obese. While the intestines of both lean and obese mice were dominated by the same two major types of bacteria (*Bacteroides* and *Firmicutes*), the relative proportion of these types was different between the lean and obese mice. Subsequently, the researchers went on to show that the bacterial population in obese mice does indeed have an increased ability to extract energy from the diet. By analyzing the collective bacterial genomes, or microbiome, of lean and obese mice, they found that the microbiome of obese mice was enriched in

STORY OF DISCOVERY

energy-extracting metabolic enzymes. Additionally, when they transplanted bacterial communities from the intestines of obese mice into germ-free mice, the recipient mice (no longer germ-free) had a greater increase in total body fat compared to mice receiving transplants of bacteria from the intestines of lean mice. These ground breaking results supported a link between the composition of intestinal bacteria and obesity.

Having discovered important connections between intestinal bacteria and obesity in mice, Dr. Gordon's team has gone on to show a similar correlation in humans. In an initial study of people who are obese, the scientists found that the human intestine is dominated by *Bacteroides* and *Firmicutes* (the same types of bacteria found in mice). More importantly, the relative proportions of these types of bacteria could be modulated by weight loss through fat- or carbohydrate-restricted diets. Although the intestinal bacteria fall into these two broadly defined types, the breadth of species variation within each type is much wider among individual people. To further explore this diversity, Gordon and coworkers have most recently performed a sequencing tour-de-force of intestinal microbiomes from lean and obese twins. Despite the variation in the bacteria found in different individuals, they found a common, or "core," set of bacterial genes that are involved in various metabolic functions. Interestingly, when comparing microbiomes, the researchers found that obesity was associated with a variation in bacterial genes involved in nutrient metabolism that may alter the energy balance in obesity.

Dr. Gordon's research provides a compelling link between the composition of bacterial genes in the intestine and excess body weight. In fact, if what was observed in mice turns out to reflect what happens in humans, Dr. Gordon's experiments with germ-free mice support a causal role for the intestinal microbial community in obesity. Additional factors might also affect the bacterial composition or their ability to extract energy from the diet to predispose an individual to obesity. In an effort to identify other potential contributing or mitigating factors, researchers are now exploring the relationships between host genetics, bacterial composition, and weight gain and obesity. While researchers pursue further advances, the current state-of-the-science offers exciting ideas about how probiotic therapies may eventually be developed to target and manipulate the resident bacteria of the intestines for the treatment and prevention of obesity. Together, research that leads to a better understanding of how nutrient metabolism is affected by intestinal bacteria and to therapeutic applications designed to target them may ultimately help reduce the public health burden associated with obesity and other conditions.

PATIENT PROFILE

Cynthia Griggs

A Case of Acetaminophen Overdose Triggering Acute Liver Failure

Cynthia Griggs

In February 2005, Cynthia Griggs was rushed to the hospital. A few days earlier she had come down with a nasty flu virus that had her vomiting and sweating profusely. She also had a high fever. To ease the severe aches and pains that accompanied her flu, Cynthia began taking a drug that many take in her situation, a remedy containing the pain-relief ingredient acetaminophen. Acetaminophen is the generic name of a drug found in many commonly used products available over-the-counter such as Tylenol® and Tylenol® PM, nonprescription cough and cold products such as Nyquil® and Theraflu®, as well as prescription products such as Vicodin and Percocet.

As the flu progressed, Cynthia, now age 46, recalls how she was so sick and in pain that she started popping acetaminophen tablets whenever she felt she needed them—without regard to the daily recommended dosage printed on the bottle. "I was in such pain and perspiring so much I could hear the beads of my sweat dropping on the floor." Her bed sheets were so soaked with perspiration that she was forced to continually switch beds.

It wasn't long after Cynthia started taking the acetaminophen tablets that her skin started to turn yellow, a sign of jaundice indicating damaged liver function. "My mother wanted to take me to the doctor, but I told her I was fine. It was just the flu," explains Cynthia. The next day, still very sick and in pain, Cynthia took even more acetaminophen in the form of a cold medicine. That's when she started to become dizzy and delusional. This time her mother immediately called for an ambulance.

Though neither she nor her mother knew it at the time, Cynthia was experiencing acetaminophen toxicity, which was causing her liver to shut down in a manner that was life-threatening.

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A Frightening Episode

Cynthia arrived at the hospital in liver and kidney failure which contributed to her acting incoherently. "The doctors told my mother there was nothing they could do. My liver was in such bad shape that I only had a 30 percent chance of surviving the night."

PATIENT PROFILE

But at some point in a conversation with the medical staff, Cynthia's mother happened to mention how much acetaminophen-containing medication Cynthia had taken in a relatively short period of time. Recognizing the danger that an excessive dose of acetaminophen can cause, the staff immediately injected Cynthia with N-acetylcysteine, or NAC, which, if administered within 12 hours of an acetaminophen overdose, serves as an antidote to the drug's toxicity. By the next morning, Cynthia was feeling much better. Both her liver and kidneys were beginning to function normally, and 2 days later she was discharged from the hospital.

"It was an overnight episode. The antidote saved my life," Cynthia says. But not everyone is as fortunate as she.

"The antidote saved my life," Cynthia says. But not everyone is as fortunate as she.

Taking more acetaminophen than the recommended dosage on its own can be extremely toxic to the liver, but other factors can exacerbate this toxicity. Cynthia's experience with acetaminophen had a potential additional complication, in that she had an enlarged liver, likely due to a period of increased alcohol use several years earlier. Her liver condition may have contributed to the damaging effects of the excessive amount of acetaminophen taken for her flu, which resulted in her hospitalization with what turned out to be acute liver failure.

Acetaminophen-Related Acute Liver Failure Increasing in the U.S.

The drug acetaminophen, contained in many commonly used medications, happens to be the leading cause of liver failure in the U.S. Although relatively rare, acute liver failure due to drugs, especially from acetaminophen, has occurred with increasing frequency in the U.S. in recent years, based on findings by researchers in the NIDDK's Acute Liver Failure Study Group. Although most cases of liver injury from medications are mild and resolve quickly, some individuals develop liver injury so severe that it can lead to acute liver failure and, ultimately, death.

Other than the antidote given to Cynthia, the only treatment currently available for acute liver failure is liver transplantation, for which donor organs are in short supply. According to the U.S. Organ Procurement and Transplantation Network, currently, the number of individuals on the waiting list for a liver transplantation in the U.S. is approximately 16,000, while only about 6,300 liver transplants were performed in the U.S. in 2008, the most recent year for which complete data are available.

The majority of acetaminophen-related deaths are due to taking excessive amounts of prescription medications. U.S. Food and Drug Administration (FDA) data indicate that overdoses of nonprescription cough and cold products that contain acetaminophen occur less frequently. However, a common scenario in which overdoses do occur is when people combine these medications with other acetaminophen-containing products and unknowingly increase their ingested dose of the drug.

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Making Acetaminophen-Containing Medications Safer

Many people take acetaminophen-containing products daily to relieve pain from chronic conditions such as arthritis, or they regularly take

products containing acetaminophen that help them sleep. When taken as directed, these products are considered to be safe. However, their safety depends on heeding the maximum daily dosage of acetaminophen—something that can be difficult to calculate when people take a combination of medications that contain acetaminophen or when they are also dealing with another liver condition. Most people do not realize that the ingredient acetaminophen is present in so many different, commonly used over-the-counter remedies, and they may thus inadvertently expose themselves to excessive amounts of acetaminophen and subsequently suffer liver failure. In fact, if a person is taking multiple acetaminophen-containing drugs, then even less than the maximum dose of each could, over the course of a day, still add up to an overdose of acetaminophen. This underscores the importance of checking medicine labels—formulations for both adults and children—for dosing, warnings, and other information, including the ingredients that are in each medicine.

In June 2009, an FDA advisory committee, made up of scientists, doctors, and consumer representatives, recommended lowering the maximum dosage of over-the-counter drugs containing acetaminophen and making high-dose acetaminophen available by prescription only. The FDA also recommended eliminating prescription acetaminophen-combination painkillers, and adding a "black-box" warning to acetaminophen-containing prescription drugs. These recommendations are intended to alert consumers to the potential for liver damage due to unintentional overdose from the drug.

Individuals who take acetaminophen-containing medications as directed, are not taking any other prescription pain medications, and do not have existing liver disease should not be at risk of damaging their livers. Alternative pain relievers such as aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) are effective, but they too can cause serious side-effects: when taken at high doses and for prolonged periods—especially for the treatment of chronic pain in older adults—they carry a risk of stomach ulcers and gastrointestinal bleeding. Those concerned about acetaminophen or other pain remedies should ask their doctor about which over-the-counter medicine is best for them.

Researching New Ways To Reduce Drug-Induced Acute Liver Failure

Research supported by the NIDDK has played an integral role in advancing knowledge about liver injury and acute liver failure caused by drugs such as acetaminophen. For example, the NIDDK-sponsored Adult Acute Liver Failure Study Group, founded in 1997, was based on investigator-initiated efforts to address this problem by expanding knowledge about natural history, causes, and outcomes of acute liver failure in the U.S. The Group has collected samples and data needed to conduct retrospective as well as forward-looking studies that more closely examine the problem of acute liver failure in the U.S., focusing largely on cases caused by drugs. The Group conducts clinical research at 24 sites throughout the country and is headed by Dr. William Lee at the University of Texas Southwestern Medical Center at Dallas, where Cynthia received treatment for her acetaminophen-related acute liver failure.

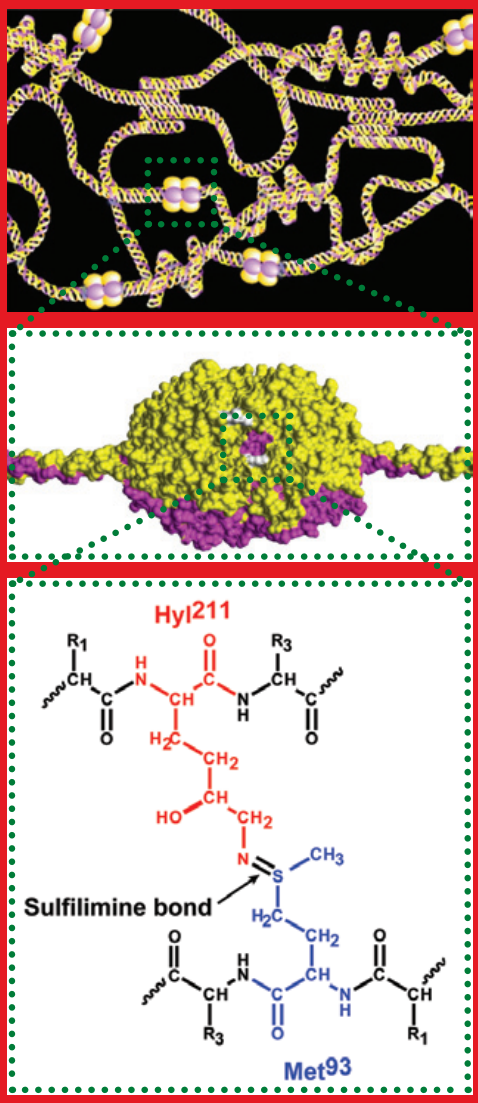
In 2002, the Adult Acute Liver Failure Study Group published the ground-breaking and alarming finding that liver injury due to acetaminophen use had risen dramatically in recent years to become the most frequent known cause of acute liver failure in the U.S. Building on this important observation, the Group developed an assay to directly identify cases of acetaminophen-induced acute liver failure by measuring unique compounds in the blood—an advance that could facilitate diagnosis and allow more accurate estimates of prevalence. In 2005, the Group expanded its focus to study the problem in children. The Pediatric Acute Liver Failure Study Group and the Adult Study Group are currently focusing on testing

PATIENT PROFILE

whether use of the antidote NAC, which can improve outcomes in cases of acetaminophen toxicity, could be expanded to cases of acute liver failure due to other causes besides acetaminophen. Recently, the Adult Acute Liver Failure Study Group published encouraging findings in the journal *Gastroenterology* showing that NAC treatment also improves survival of patients with acute liver failure due to other causes, especially in patients in the earliest stages of acute liver failure.

Another NIDDK research effort in this area, the Drug-Induced Liver Injury Network, was established in 2003 to collect and analyze cases of severe liver injury caused by prescription drugs, over-the-counter drugs, and alternative medicines, such as herbal products and supplements. This Network of five clinical centers and one data coordinating center aims to develop better tools for directly diagnosing, and ultimately preventing, drug-induced liver injury, as well as enhancing knowledge of disease processes. The Network is currently conducting a retrospective study to establish a nationwide registry of people who experienced liver injury within the past 10 years after using one of seven specific drugs or a drug from a specific category of antibiotics. A prospective study is underway to form a nationwide registry of people who experienced liver injury after using certain drugs or alternative medicines. Also, in conjunction with the National Library of Medicine, the NIDDK is developing a Web site to release in 2010 that will feature sample cases of drug-induced liver injury based on Network data, as well as a database summarizing reports of liver injury for a given drug. This Web site will serve as a resource to aid health care providers in diagnosing, and investigators in studying, liver injury due to drugs.

Patients such as Cynthia can benefit from these and other NIDDK-supported research studies directed toward goals addressing drug- and toxicant-induced liver disease in the trans-NIH *Action Plan for Liver Disease Research* (*http://liverplan.niddk.nih.gov*). These research efforts are helping to alleviate the problem of acute liver failure caused by drugs such as acetaminophen and contribute to potentially life-saving care.



Researchers have recently discovered a new type of chemical bond in collagen IV proteins (model depicted in upper panel). Collagen IV proteins form a network that provides the structural integrity of the glomerular basement membrane. The collagen IV network is stabilized by crosslinks or bonds between different collagen IV proteins. It was known that a bond was established between two of the building blocks of proteins: a methionine amino acid (Met) and a modified lysine amino acid called hydroxylysine (Hyl). However, the specific nature of the bond remained elusive, despite decades of investigation. Recently, using cutting-edge instrumentation, the scientists discovered that these collagen IV components are linked together with a "sulfilimine bond" (bottom panels). This type of chemical bond had never before been identified in a biological specimen; scientists speculate that it may add strength to the collagen IV network. For more on this research advance and how it relates to an autoimmune kidney disease called Goodpasture's syndrome, please see the write-up later in the chapter.

Graphics provided by Dr. Billy G. Hudson from Vanacore R, Ham A-JL, Voehler M, Sanders CR, Conrads TP, Veenstra TD, Sharpless KB, Dawson PE, and Hudson BG: A sulfilimine bond identified in collagen IV. Science 325: 1230-1234, 2009. Reprinted with permission from AAAS.

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the U.S. They afflict millions of Americans, including children and young adults. The NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The goal is to increase understanding of kidney, urologic, and hematologic diseases to enhance prevention and treatment strategies.

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, even for a short period of time or due to gradual deterioration, can result in life-threatening complications. Whether kidney function is lost suddenly or slowly represents an important health challenge.

Chronic kidney disease has two main causes: high blood pressure and diabetes. Recent estimates put the number of Americans with chronic kidney disease at more than 23 million.[1] If unchecked, the recent increases in obesity and type 2 diabetes in the U.S.—especially among children and adolescents—have grave implications, as individuals are likely to face any secondary health consequences at an earlier age than people who develop these conditions as middle-aged adults.

Chronic kidney disease, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. At the close of 2007, more than 525,000 patients were receiving treatment for ESRD: nearly 370,000 were undergoing dialysis and almost 160,000 were living with a kidney transplant. Racial minorities, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease and ESRD. African Americans are four times more likely and American Indians are twice as likely to develop kidney failure as non-Hispanic whites. Hispanics have a significantly increased risk for kidney failure as well.[2]

The NIDDK supports a significant body of research aimed at understanding the biology underlying chronic kidney disease. The Institute's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease; the underlying mechanisms leading to progression of kidney disease to ESRD; and the identification and testing of possible treatments to prevent development or halt progression of kidney disease. Also of interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related kidney diseases such as IgA nephropathy and hemolytic uremic syndrome. The Institute's National Kidney Disease Education Program is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat chronic kidney disease and prevent kidney failure. It represents a major educational outreach effort to patients, physicians, and the public.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research effort includes basic, clinical, and epidemiologic research on the genitourinary tract.

Benign prostatic hyperplasia (BPH) is an enlargement of the prostate—a gland below the bladder that surrounds the urethra—a common condition affecting about 50 percent of men in their 50s. When enlarged, the prostate can restrict urine flow from the bladder. Lower urinary tract symptoms (LUTS) are the

[1] *Levey AS, et al: Ann Intern Med 150: 604-612, 2009.*

[2] *U.S. Renal Data System, USRDS 2009 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the U.S., National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2009.*

symptoms thought to be related to BPH; however LUTS are not exclusive to BPH or men.

Infections of the urinary tract are extremely common in women, and many women suffer repeated urinary tract infections (UTIs). Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating, chronic, and painful bladder disease. IC/PBS affects both men and women, but it is nine times more common in women. In men, prostatitis—chronic, painful inflammation of the prostate gland—accounts for a significant percentage of all physician visits by young and middle-aged men for complaints involving the genitourinary system.

NIDDK-supported basic and clinical research is focused on elucidating the causes of IC/PBS, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. The Interstitial Cystitis Clinical Trials Group/Research Network conducts clinical studies in IC/PBS. NIDDK's Multidisciplinary Approach to the Study of Urologic Chronic Pelvic Pain (MAPP) Syndromes Research Network supports studies designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients. The goals and approaches of the MAPP Research Network reflect the most current thinking on IC/PBS pathology and involve significant new advancements in how IC/PBS is studied. All efforts are designed to provide insights that can be translated to improve the clinical care of IC/PBS patients.

A conservative estimate is that approximately 13 million Americans, most of them women, suffer from urinary incontinence.[3,4] Many suffer in silence due to embarrassment and lack of knowledge about options available. The introduction of new surgical procedures has advanced the treatment of urinary incontinence dramatically in the last decade. The NIDDK's Urinary Incontinence Treatment Network recently completed the Trial of Mid-Urethral Slings (TOMUS) study comparing two minimally invasive surgeries for the treatment of stress urinary incontinence and results are expected in 2010.

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and chronic disease. The Institute is also keenly interested in the basic biology of stem cells, including adult hematopoietic stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the Institute's hematology research program is the development of improved iron chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

DELVING INTO COLLAGEN NETWORKS

A New Chemical Bond in Collagen IV Networks: Researchers have discovered a new kind of chemical bond in biological tissue, a fundamental discovery in structural biology that may provide insights into several human diseases. The bond connects two of the subunits that make up collagen IV, a protein that is an important component of the extracellular matrix. Collagen provides structural support to tissues, serves as a scaffold upon which other complexes are assembled, and mediates cellular signaling. Scientists have long known that collagen subunits are linked to one another through intermolecular bonds that lend strength and structural integrity to the matrix; however, the precise nature of these bonds had eluded them. Using advanced techniques to study protein structure, the researchers discovered a novel chemical bond between a sulfur atom of the amino acid methionine on one subunit and a nitrogen atom of a modified form of the amino acid lysine on another. This is the first time that a "sulfilimine" bond—a direct bond between sulfur and nitrogen atoms—has been found in a native biomolecule.

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[3] *Nygaard I, et al: Urinary Incontinence in Women in Urological Diseases in America (pp. 157-191). NIDDK, NIH Publication Number 07-5512, 2007.*

[4] *Stothers L, et al: Urinary Incontinence in Men in Urological Diseases in America (pp. 193-221). NIDDK, NIH Publication Number 07-5512, 2007.*

Collagen IV networks have been implicated in a number of human diseases. Two collagen subunits are defective in the inherited kidney disorder Alport syndrome, a condition in which waste filtering by the kidney is impaired due to disruption of the extracellular matrix. Furthermore, one collagen chain is involved in the rare, autoimmune kidney disease, Goodpasture's syndrome. In this disease, the misguided antibody attack on the kidney's extracellular matrix is thought to be due to exposure of a usually hidden part of the collagen molecule, which may be made accessible as a consequence of the absence or breakage of the sulfilimine bond. Discovery of this novel chemical bond therefore represents not only an important advance in our knowledge of collagen structure, but also may identify causes of disease and possible treatment approaches.

Vanacore R, Ham AJ, Voehler M, Sanders CR, Conrads TP, Veenstra TD, Sharpless KB, Dawson PE, Hudson BG: A sulfilimine bond identified in collagen IV. Science 325: 1230-1234, 2009.

GENETICS OF KIDNEY DISEASE

Common Genetic Variants as Contributors to Risk of Chronic Kidney Disease: Researchers have identified variations at five distinct genetic regions (loci) that are associated with either diminished kidney function or chronic kidney disease. The scientists performed genome-wide association scans on nearly 20,000 biological samples that had been collected from volunteers during previous trials, and then replicated these results with a separate set of over 21,000 additional specimens. They found that variations in the *UMOD* genetic locus were associated with chronic kidney disease; that variations at the *UMOD*, *SHROOM3*, and *GATM-SPATA5L1* loci were associated with diminished kidney filtration rates as determined by mathematically extrapolating from serum creatinine levels; and that variations at the *CST* and *STC1* loci were associated with diminished kidney filtration rates extrapolated from serum cystatin C levels.

Several of these genes are likely to play a direct role in kidney function. The *UMOD* gene encodes the most abundant protein found in the urine of healthy people, although its biological function is unclear. The *SHROOM3* gene product is present in human kidneys and is thought to play a role in the regulation of cell shape. And the *SCT1* gene product may help regulate calcium and phosphate balance. The discovery and validation of several common variants at previously unidentified genetic regions that are associated with increased risk of diminished kidney function and chronic kidney disease have important implications for both researchers and clinicians. Further studies to understand the role of these proteins in kidney disease—including discovering the function of the protein encoded by the *UMOD* gene—may lead to new approaches to prevent and treat chronic kidney disease.

Köttgen A, Glazer NL, Dehghan A, Hwang SJ, Katz R, Li M, Yang Q, Gudnason V, Launer LJ, Harris TB, Smith AV, Arking DE, Astor BC, Boerwinkle E, Ehret GB, Ruczinski I, Scharpf RB, Chen Y-DI, de Boer IH, Haritunians T, Lumley T, Sarnak M, Siscovick D, Benjamin EJ, Levy D, Upadhyay A, Aulchenko YS, Hofman A, Rivadeneira F, Uitterlinden AG, van Duijn CM, Chasman DI, Paré G, Ridker PM, Kao WH, Witteman JC, Coresh J, Shlipak MG, and Fox CS: Multiple loci associated with indices of renal function and chronic kidney disease. Nat Genet 41: 712-717, 2009.

KIDNEY FIBROSIS RESEARCH

Identification of the Cellular Source of Scar-Producing Collagen in a Model of Kidney Fibrosis: Scientists have recently pinpointed a type of cell in the kidney that appears to play a major role in tissue scarring that is seen in some forms of kidney disease. Fibrosis is the term that describes the deposition of large amounts of collagen-rich connective tissue that can lead to scarring within an organ. It is seen in many conditions related to inflammation and, unchecked, can diminish the ability of an organ to perform its normal functions. In the kidney, fibrosis can impair the removal of toxins and excess fluid from the blood, cause irreversible kidney damage and, in extreme cases, lead to kidney failure. Therefore, the causes of fibrosis are of great interest to researchers and physicians.

To study the molecular and cellular biology of fibrosis in the kidney, researchers used mice in which cells that produce a particular form of collagen were "tagged" with a fluorescent protein, allowing them to be clearly

identified. They then used surgical obstruction of one ureter—the conduit through which urine collected by the kidney flows to the bladder—to induce kidney inflammation and subsequent fibrosis. Following ureteral obstruction, cells known as myofibroblasts were found to be the major source of collagen in the fibrotic kidneys. Interestingly, the vast majority of myofibroblasts in the kidney were derived from pericytes, cells that have the potential to differentiate into other cell types and are often found associated with the walls of small blood vessels, including those that pass through the kidney. The identification of pericytes, which are not derived from the kidney's nephrons but rather from its associated vasculature, as the source of collage-producing myofibroblasts will likely focus renewed attention on the role of the circulatory system in triggering and/or mediating kidney fibrosis. Understanding the origin of the scar-producing cells is a key step in elucidating the mechanisms through which fibrosis develops in response to kidney injury or inflammation, and may identify new targets for future therapeutic interventions.

Lin S-L, Kisseleva T, Brenner DA, and Duffield JS: Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. Am J Pathol 173: 1617-1627, 2008.

AUTOIMMUNE KIDNEY DISEASE

Potential Immune System Target Antigen in Autoimmune Kidney Disease: Researchers have identified a protein that may play a key role in the development of an autoimmune form of kidney disease known as "idiopathic membranous nephropathy." This type of kidney disease is a common cause of nephrotic syndrome in adults. It is an autoimmune disease, meaning that the body's immune system incorrectly mounts an attack against a normally occurring protein in the body. The disease is characterized by protein in the urine, lowered protein levels in the blood, elevated cholesterol, and swelling of the face, hands, and feet. The identification of the protein that induces this immune response (termed an "antigen") will open new avenues of exploration in idiopathic membranous nephropathy.

To identify potential target antigens, scientists collected blood samples from patients with idiopathic membranous nephropathy, and mixed them with proteins that were obtained from kidney tissue. In 70 percent of the tested blood samples, self-reactive antibodies (autoantibodies) identified a single kidney protein that was ultimately determined to be the M-type phospholipase A_2 receptor or PLA_2R. This protein is expressed by cells in glomeruli, tiny filtering units in the kidney that are injured in this syndrome. The subtype of antibody that reacted with PLA_2R in the assay is the same kind that is found in immune deposits within the glomeruli in patients with this disease. Antibodies isolated from glomeruli of patients with idiopathic membranous nephropathy react with PLA_2R, whereas antibodies isolated from the glomeruli of patients with nephropathy arising from other causes do not. Furthermore, there is evidence to suggest that, in patients with clinically significant disease activity, autoantibodies against PLA_2R can be readily detected in the blood. In contrast, in patients in whom the disease is in remission, levels of these antibodies decline or disappear.

Fifty years ago, researchers studying a rat model of idiopathic membranous nephropathy identified a kidney protein that appeared to be an immunological target for autoantibodies; however, progress stalled when this protein was found to be absent in human kidneys. These new findings will also have important implications for patient care. For example, they may permit the noninvasive diagnosis of membranous nephropathy, as well as provide an easier way to follow the disease in response to treatment. Better understanding of the potential triggers of autoantibody production in patients with a susceptibility to idiopathic membranous nephropathy may also uncover possible new targets for preventing or treating this disease.

Beck LH Jr, Bonegio RGB, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, and Salant DJ: M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med 361: 11-21, 2009.

New Insight into the Immunology of Goodpasture's Syndrome: Scientists have recently reported new findings on the immunological events relevant to a debilitating autoimmune disease known as Goodpasture's syndrome (GPS). This disease is a rare condition marked by kidney damage—sometimes

leading to kidney failure—and bleeding in the lungs. Its precise underlying cause is unknown but, as with all autoimmune diseases, it arises when the cells of the immune system wrongly recognize "self" molecules as foreign and initiate an attack on them. Under normal circumstances, developing immune cells that react with proteins that occur naturally in the body are selectively deleted. For example, the B cells of the immune system produce antibodies to attack invaders such as infectious agents. However, if a particular B cell's antibodies happen to react to one of the body's own proteins, that B cell is either eliminated, altered to produce different antibodies, or otherwise neutralized. This process is known as "tolerance." It has not been clear why people with Goodpasture's syndrome, or other autoimmune diseases, have self-reactive antibodies. One theory is that, in people with autoimmune diseases such as GPS, potentially self-reactive immune cells are not deleted as they should be. Another theory is that non-self-reactive immune cells may undergo chromosomal rearrangement, rendering them self-reactive.

To explore how self-reactive antibodies in Goodpasture's syndrome could escape tolerance-induced deletion, a group of researchers generated a mouse model to study based on knowledge of the target of these antibodies. In previous research, scientists had identified a region of the alpha3 chain of type IV collagen—a component of connective tissue present in the kidneys and the lungs—as the target of this immune attack. This target is normally hidden amid the other portions of collagen, and exists only in a few body tissues. Thus, it was speculated that this region of collagen may not be sufficiently available to developing B cells for the body to screen out any that produce reactive antibodies. In Goodpasture's syndrome, it was thought that perhaps this collagen target later becomes aberrantly available to the B cells, after the normal time at which tolerance occurs, resulting in an antibody reaction and tissue damage. In the current study, researchers engineered mice with B cells producing antibodies that recognize the same region of collagen as the immune cells in people with GPS. Surprisingly, the researchers found that the B cells producing self-reactive antibodies were selectively eliminated in the bone marrow, before they had a chance to circulate throughout the body. The researchers then investigated whether any of the B cells might have altered their antibody structures so they would no longer react against collagen. To assess this possibility, they compared antibodies in these mice and in mice lacking a gene, called *Rag*, which is required for this type of antibody alteration. They found that some of the formerly self-reactive B cells appeared to have modified their antibodies; mice deficient in the *Rag* gene did not have these altered antibodies.

These findings overturn previous speculation about the origin of self-reactive antibodies in Goodpasture's syndrome. The study suggests that, in most individuals, auto-reactive B cells are either eliminated or sufficiently modified in the bone marrow during maturation. The development of Goodpasture's syndrome may thus result from either the escape of a few collagen-reactive immune cells, perhaps accompanied by other disease triggers, or from changes to B cells following self-tolerization.

Zhang Y, Su SC, Hecox DB, Brady GF, Mackin KM, Clark AG, and Foster MH: Central tolerance regulates B cells reactive with Goodpasture antigen alpha3(IV)NC1 collagen. J Immunol 181: 6092-6100, 2008.

POTENTIAL CAUSE OF REDUCED KIDNEY FUNCTION AND KIDNEY STONE FORMATION

Genome-Wide Scan Identifies Genes Linked to Increased Risk of Gout: Researchers have recently reported the identification of two new genes and confirmed the previous finding of a third gene associated with increased levels of uric acid in the blood and increased risk of gout, a form of arthritis. Chronic gout is associated with increased risk of kidney stone formation and decreased kidney function. Among the known risk factors for gout, hyperuricemia is a condition in which there is an excess level of uric acid in the blood. Under circumstances that are not yet completely understood, the excess uric acid begins to crystallize, causing inflammation and pain in the joints. Previous studies have identified a type of variation in DNA sequence, called single nucleotide polymorphisms (SNPs), in the *SLC2A9* gene that correlated with increased levels of uric acid and gout.

Researchers sought to identify additional genes that contribute to increased uric acid levels and gout. They conducted genome-wide association studies on samples from participants of several large studies—7,699 participants in the well-characterized Framingham Heart Study and 4,148 from the Rotterdam Study—to first identify genetic regions associated with increased uric acid levels. They then confirmed those findings in samples from 14,867 participants in another study, the Atherosclerosis Risk in Communities study. Subsequent analysis determined whether SNPs associated with increased uric acid levels are linked to the development of gout. Two new genes, *ABCG2* and *SLC17A3*, and the previously identified gene *SCL2A9*, were shown to contain SNPs that were associated with both increased uric acid levels and gout. All three of these genes code for proteins suspected of transporting uric acid and potentially other small molecules across the kidney cell membrane. Interestingly, the SNPs for *SCL2A9* and *ABCG2* were associated with increased uric acid levels in both white and African American participants, whereas the variant for *SLC17A3* correlated only with increased uric acid levels in whites.

The investigators then determined whether these gene variants were associated with the development of gout. In contrast to the SNP for *ABCG2*, which was associated with gout in both white and African American participants, the variants for *SCL2A9* and *SLC17A3* were associated with gout in white participants only.

This study identifies additional genes having a role in uric acid buildup and development of gout. It also reinforces the concept that gene variants may act differently on different genetic backgrounds. This knowledge may lead to new treatment strategies as well as the development of drugs with fewer side effects compared to those currently available.

Dehghan A, Köttgen A, Yang Q, Hwang S-J, Kao WHL, Rivadeneira F, Boerwinkle E, Levy D, Hofman A, Astor BC, Benjamin EJ, van Duijn CM, Witteman JC, Coresh J, and Fox CS: Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. Lancet 372: 1953-1961, 2008.

DEVELOPING NEW TREATMENTS FOR KIDNEY DISEASE

Combination of Aspirin and an Anti-Clotting Drug Reduces Risk of Dialysis Access Failure:

For the first time, a combination of aspirin and the anti-platelet drug dipyridamole has been shown to significantly reduce blockages and extend the useful life of new artery-vein (AV) access grafts used for hemodialysis, in a study conducted by the NIDDK-supported Dialysis Access Consortium (DAC). Patients undergoing dialysis for kidney failure sometimes have a synthetic tube, or graft, implanted under the skin of the arm. The graft becomes an artificial vein that can be used repeatedly for needle placement and blood access during hemodialysis. When these access grafts fail, it is most often due to narrowing of blood vessels at the graft site (termed "stenosis") and subsequent clotting, both of which can diminish or block the flow of blood. A blocked graft cannot be used for dialysis and is a major cause of deteriorating health in dialysis patients.

The DAC trial enrolled a total of 649 participants with new AV grafts who were randomly assigned to receive either aspirin and dipyridamole or a placebo. Both aspirin and dipyridamole inhibit platelet aggregation, which can result in the formation of blood clots. The trial found that the combination drug treatment decreased the rate of loss of the useful life of a graft before it becomes blocked the first time by 18 percent, and the rate of developing significant stenosis by 28 percent, compared to placebo, modestly but significantly prolonging the viability of AV grafts. However, the overall rates of graft failure, complications such as bleeding, and death were similar in both groups. This finding validates an approach that may help to maintain AV grafts so that patients can continue to receive life-sustaining dialysis. It is also a step forward in the development of therapies that improve the quality of life for dialysis patients. In order to improve long-term graft viability, future studies may address the underlying causes of vascular stenosis at the graft site.

Dixon BS, Beck GJ, Vazquez MA, Greenberg A, Delmez JA, Allon M, Dember LM, Himmelfarb J, Gassman JJ, Greene T, Radeva MK, Davidson IJ, Ikizler TA, Braden GL, Fenves AZ,

Kaufman JS, Cotton JR Jr, Martin KJ, McNeil JW, Rahman A, Lawson JH, Whiting JF, Hu B, Meyers CM, Kusek JW, Feldman HI, for the DAC Study Group: Effect of dipyridamole plus aspirin on hemodialysis graft patency. N Engl J Med 360: 2191-2201, 2009.

Potential New Therapy for Patients with the Kidney Disease Nephrogenic Diabetes Insipidus: NIH scientists have recently developed the first viable animal model of the kidney disease nephrogenic diabetes insipidus (NDI) and have shown that drug treatment can greatly reduce the manifestations of the disease. The vast majority of patients with NDI have a mutation resulting in loss of function of the V2 vasopressin receptor (*V2R*) gene. Because the *V2R* gene is located on the X chromosome, this form of NDI is called X-linked NDI or XNDI and, as with other X-linked diseases, it is seen almost exclusively in males. In people with XNDI, the kidneys are unable to effectively reabsorb water during filtration, leading to the excretion of large volumes of dilute urine, which can result in electrolyte imbalance and dehydration. In severe cases, patients are at risk for kidney damage and failure, mental retardation, or—in undiagnosed infants and children—failure to thrive and death. Currently, there is no effective treatment for XNDI, and research has been hampered by the absence of good animal models in which to study the disease and to test potential therapies.

NIDDK intramural scientists, in collaboration with National Heart, Lung, and Blood Institute scientists, developed a mouse model in which the *V2R* gene could be selectively deleted in adult animals. Deletion of this gene resulted in the appearance of disease symptoms that very closely resembled human XNDI. The mice excreted large amounts of dilute urine, did not gain weight as fast as normal mice, and developed kidney damage. With a viable mouse model of XNDI that has all the key symptoms of the human disease, the scientists next turned to evaluating possible treatments. The V2R protein transmits signals within the cell through mediators known as G proteins, which act to increase intracellular levels of a molecule called cyclic AMP. Administration of a drug that activates the prostaglandin E receptor 4 (EP4), which also acts through G proteins and cyclic AMP, compensated significantly for the absence of V2R signaling. Mice that received a single injection of the drug showed an almost immediate decreased output of urine that was more concentrated, indicating that the drug was highly effective and quick-acting. Prolonged drug treatment was not associated with any obvious side effects. Moreover, kidney damage was halted in mice during extended drug treatment, while it worsened over the same period in animals that did not receive the drug. Together, these observations demonstrate that signaling through the EP4 receptors may represent a valid approach to ameliorate the symptoms of XNDI. These findings should stimulate the development of a new generation of drugs to treat this form of human nephrogenic diabetes insipidus.

Li JH, Chou CL, Li B, Gavrilova O, Eisner C, Schnermann J, Anderson SA, Deng CX, Knepper MA, Wess J: A selective EP4 PGE2 receptor agonist alleviates disease in a new mouse model of X-linked nephrogenic diabetes insipidus. J Clin Invest 119: 3115-3126, 2009.

KIDNEY TRANSPLANT RESEARCH

Living Kidney Donors Have Similar Long-Term Survival and Quality of Life as the General Population: Most kidney transplants involve organs from cadaveric donors; however, the demand for these organs far exceeds their supply. Therefore, some people opt to donate one of their two healthy kidneys, often to a sibling or other relative whose kidneys have failed. A recent study of nearly 3,700 people who donated kidneys between 1963 and 2007 found that people who choose to donate a kidney appear to have a normal life span, and that their risk of developing kidney failure is similar to that of the general population. Within a subset of donors who were studied more intensively, kidney function and high blood pressure were similar to the general population, and their quality of life was found to be excellent. Importantly, the researchers found no evidence of excess loss of kidney function over time in donors, some of whom donated kidneys 20 or more years ago.

Although the results of this study are good news for the participants, there are some important caveats. Potential kidney donors must meet strict selection

criteria before they are allowed to donate. The relatively good health of the donors may explain at least part of the reason why their health and quality of life was found to be at least equal to, or better than, that of the general population. Additionally, in the past many volunteers donated a kidney at a relatively young age. In more recent times, however, the average age of donors has risen, and researchers and physicians will need to carefully monitor the health of these older volunteers. Additionally, participants in the current study were overwhelmingly Caucasian, and researchers do not know to what extent these findings can be extrapolated to kidney donors of other races and ethnicities. For example, African Americans have a much higher rate of diabetes, high blood pressure, and kidney failure than Caucasians, and it is possible they might be more likely to develop those conditions after donating a kidney. Further research will be necessary to better understand the long-term implications of kidney donation across a broad spectrum of the American population.

Nevertheless, the results of the current study indicate that there are few or no long-term detrimental health consequences for individuals who choose to donate a kidney. This finding may make potential donors more likely to donate a kidney, and have the consequence of increasing the supply of organs available for transplant.

Ibrahim HN, Foley R, Tan L, Rogers T, Bailey RF, Guo H, Gross CR, and Matas AJ: Long-term consequences of kidney donation. N Engl J Med 360: 459-469, 2009.

UROLOGY RESEARCH

Weight Loss in Overweight and Obese Women Reduces Urinary Incontinence: Researchers have recently reported that weight loss reduces urinary incontinence in overweight and obese women. An estimated 13 million Americans, most of them women, suffer from urinary incontinence. Women usually experience either "stress" and/or "urge" urinary incontinence. Stress urinary incontinence is the leakage of small amounts of urine during physical activity, such as coughing, sneezing, and exercising. Urge urinary incontinence is the leakage of large amounts of urine at unexpected times, including during sleep. Many women who have the disorder suffer in silence due to embarrassment. Obesity is an established and modifiable risk factor for urinary incontinence, but conclusive evidence for a beneficial effect of weight loss on urinary incontinence has been lacking.

The NIDDK's Program to Reduce Incontinence by Diet and Exercise (PRIDE) study recruited 338 obese and overweight women, who leaked urine at least 10 times per week, to determine whether a weight loss program could significantly reduce the frequency of urinary incontinence. The women were randomly assigned to one of two groups—one that participated in an intensive 6-month weight loss program of diet, exercise, and behavioral modification; or another that received information about diet and exercise, but no training to help them change their lifestyle. After 6 months, the investigators reported that women in the intensive group lost an average of 8 percent of their body weight (about 17 pounds) and reduced weekly urinary incontinence episodes by nearly one-half (47 percent). In contrast, women in the information-only group lost an average of 1.6 percent of body weight (about 3 pounds) and had 28 percent fewer episodes. Among women in the intensive treatment group, 41 percent achieved a clinically important reduction of at least 70 percent of weekly total incontinence episodes, whereas 22 percent of women in the information-only group achieved the same level of reduction. PRIDE provides high-level evidence that a behavioral intervention reduces urinary incontinence in overweight and obese women thereby permitting patients and their health care providers to make better informed and personalized treatment decisions to improve this disorder.

Subak LL, Wing R, West DS, Franklin F, Vittinghoff E, Creasman JM, Richter HE, Myers D, Burgio KL, Gorin AA, Macer J, Kusek JW, and Grady D for the PRIDE Investigators: Weight loss to treat urinary incontinence in overweight and obese women. N Engl J Med 360: 481-490, 2009.

A Potential Vaccine Approach for the Treatment of Urinary Tract Infection: Scientists have recently reported the successful use of a live, attenuated vaccine in mice to prevent bacterial infection in the bladder. Bladder and urinary tract infections (UTIs) are common, especially in women. About one-third of all women in the U.S. are diagnosed with a UTI by the time they reach 24 years of age—and many women suffer repeated UTIs. Most UTIs are caused by a

common type of *Escherichia coli* (*E. coli*) bacterium. While antibiotic treatments are available, the rise of antimicrobial resistance among urinary tract invaders warrants improved prevention and treatment strategies.

The researchers hypothesized that use of attenuated (live but weakened) bacteria that stimulate the host immune response without causing disease may be beneficial in preventing or ridding the body of UTIs. A mutant strain of UTI-causing *E. coli* engineered to lack a gene important to the bacteria's virulence (*waaL*) appeared to be a strong candidate. To begin to assess whether the mutant *E. coli* may be clinically beneficial in protecting a host from UTI, the researchers performed a series of experiments testing its efficacy in rodent models. In these experiments, mutant *E. coli* were delivered into the bladders of uninfected mice as a potential vaccine; these mice, as well as mock-treated mice, were then challenged 14 days later with the normal, UTI-causing *E. coli*. The scientists found that mice inoculated with the mutant bacteria vaccine were protected from subsequent bladder infection. Importantly, the mutant bacteria were not themselves able to effectively infect the mouse bladders. Vaccination with the mutant *E. coli* strain also not only protected against infection by the original bacterial strain from which it was derived, but also protected against challenge from other, different strains of UTI-causing *E. coli*—indicating that this type of vaccine approach may provide broad clinical protection. While the exact mechanism for this protection is still under investigation, and additional studies will need to be performed to determine whether this live-attenuated vaccine may also hold promise for resolving recurrent UTIs in people, these findings are encouraging in the quest to find effective new UTI therapies.

Billips BK, Yaggie RE, Cashy JP, Schaeffer AJ, and Klumpp DJ: A live-attenuated vaccine for the treatment of urinary tract infection by uropathogenic Escherichia coli. *J Infect Dis 200: 263-272, 2009.*

Common Treatment for Chronic Prostatitis-Chronic Pelvic Pain Syndrome Fails To Reduce Symptoms: Scientists recently reported that a drug commonly prescribed for men with chronic prostatitis-chronic pelvic pain syndrome did not significantly reduce symptoms compared to a placebo control. Chronic prostatitis-chronic pelvic pain syndrome has no known cause and no uniformly effective therapy, and is the most common subtype of prostatitis seen by physicians. Men with this condition experience pain in the genital and urinary tract areas, lower urinary tract symptoms such as pain in the bladder area and during urination, and sexual problems that can severely affect their quality of life. More than one-half of primary care physicians prescribe alpha blockers, a class of drugs that relax the smooth muscle of the bladder and prostate, to treat the symptoms of chronic prostatitis-chronic pelvic pain syndrome. Some small randomized clinical trials have suggested that alpha blockers are effective in treating the symptoms of this condition. Because of the widespread use of these drugs, researchers recently examined the effectiveness of an alpha blocker in a larger randomized, placebo-controlled clinical trial in men with chronic prostatitis-chronic pelvic pain syndrome who had not previously been treated with this class of drugs.

A total of 272 men recently diagnosed with chronic prostatitis-chronic pelvic pain syndrome were randomly assigned to treatment for 12 weeks with the alpha blocker, alfuzosin, or a placebo. The NIH Chronic prostatitis-chronic pelvic pain syndrome Symptom Index score—a 0 to 43-point scale that measures severity of symptoms—was used to assess the effectiveness of alfuzosin versus placebo. The Index measures aspects of three important symptom areas associated with chronic prostatitis–chronic pelvic pain syndrome: pain, voiding problems, and negative effects on quality of life. After 12 weeks, no significant Index differences were observed between the two groups. Thus, the study showed that a drug commonly prescribed for men with chronic prostatitis–chronic pelvic pain syndrome was not effective in improving symptoms compared to placebo. The results of this study will inform future clinical trials. They may also impact patient care, sparing patients from an ineffective treatment for their condition.

Nickel JC, Krieger JN, McNaughton-Collins M, Anderson RU, Pontari M, Shoskes DA, Litwin MS, Alexander RB, White PC, Berger R, Nadler R, O'Leary M, Liong ML, Zeitlin S, Chuai S, Landis JR, Kusek JW, Nyberg LM, and Schaeffer AJ for the Chronic Prostatitis Collaborative Research Network: Alfuzosin and symptoms of chronic prostatitis-chronic pelvic pain syndrome. N Engl J Med 359: 2663-2673, 2008.

HEMATOLOGY RESEARCH

Improving Blood Stem Cell Transplantation Outcomes for Patients with Severe Sickle Cell Disease: Scientists have recently reported success with a modified bone marrow transplantation procedure to treat adult patients with sickle cell disease. Sickle cell disease is caused by a mutation in the beta globin chain of hemoglobin A, resulting in a chronic, often fatal, anemia. Under conditions of low oxygen, red blood cells become rigid and sickle-shaped, blocking blood vessels and causing severe pain. In the U.S., this genetic disease occurs predominantly in people of African descent, and is accompanied by episodic severe pain in the joints, leg ulcers, jaundice, organ damage, and other serious health conditions including, in rare cases, multi-organ failure. Mature red blood cells arise from stem cells. Transplantation of blood stem cells, obtained from bone marrow or another source such as umbilical cord blood, has been used to cure children with severe congenital anemias, such as sickle cell anemia. However, the medical procedures used for preparing patients for transplantation have thus far been too toxic to be used in adults.

A team of researchers developed a different regimen in preparation for blood stem cell transplantation, with the goal of making this treatment approach safer for use in adults. Bone marrow or other blood stem cell transplantation is typically preceded by destruction of the patient's own blood cells, to prevent immune reactions against the transplanted healthy donor cells and eliminate the disease-carrying blood cells. In this new regimen, instead of using chemotherapy to destroy the patient's bone marrow before infusing donor stem cells—as in the standard, prohibitively toxic procedure—the researchers used a low dose of radiation combined with two immunosuppressive drugs. This type of procedure is referred to as "non-myeloablative," meaning that it does not destroy the patient's own bone marrow. Rather, it is thought to create "space" for the donor stem cells to successfully engraft. After undergoing the non-myeloablative procedure, the patients, who all had severe sickle cell disease, were infused with peripheral blood stem cells from healthy sibling donors.

At a median follow-up of 2.5 years post-transplantation, the researchers reported that all 10 adult participants who had sickle cell disease were alive, and that 9 of them showed several reversed sickle cell diagnostic markers—including the return of hemoglobin levels to within normal range and the reduction of total amount of HbS protein to that seen in the donors. The results of this modified transplantation protocol strongly suggest that adult patients with sickle cell disease now have an additional option when considering how best to treat their condition.

Hsieh MM, Kang EM, Fitzhugh CD, Link MB, Bolan CD, Kurlander R, Childs RW, Rodgers GP, Powell JD, and Tisdale JF: Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. N Engl J Med 361: 2309-2317, 2009.

BCL11A Regulates Production of Fetal Hemoglobin: Two research groups recently reported that the protein BCL11A acts to repress a gene encoding one of the two forms of globin in human fetal hemoglobin (also called "HbF"). HbF consists of four globin subunits: two "alpha globin" subunits and two "gamma globin" subunits. HbF is present during human fetal development but, owing to the shut-down of the genes encoding the gamma subunits, levels of HbF begin to decline after birth. The form of hemoglobin in children and adults contains a different type of subunit, called "beta globin," instead of the gamma subunits of HbF. Although HbF is virtually undetectable in most adults, people who have hemoglobin disorders such as sickle cell disease, which causes blockage of blood vessels by red blood cells that have formed a crescent-like shape, or beta-thalassemia, which results in reduced number and viability of red blood cells, sometimes retain varying levels of HbF after birth. This HbF can partially compensate for the defective or impaired function of adult hemoglobin in sickle cell disease or thalassemia, thereby ameliorating the clinical condition. Thus, prevention or reversal of the natural HbF decline in children and adults represents a potential strategy for the treatment of these disorders.

Previous studies had identified the protein BCL11A as a possible regulator of one of the globin genes, *HBG2*, which encodes the gamma subunit of HbF. Recently two independent research groups sought to confirm BCL11A's involvement in HbF regulation and determine its mode of action. Using cell culture

systems, the scientists artificially increased or decreased the level of BCL11A protein and subsequently monitored the level of HbF or *HBG2* gene activity. High levels of BCL11A were found to decrease HbF levels. Under conditions of low to no BCL11A, HbF levels increased. Additional studies indicated that BCL11A directly acts to block HbF production by binding to specific sites near the *HBG2* gene, thereby preventing it from being turned on.

One of these research teams sought to determine the reason why humans and mice contain different globin subunits during early development. Whereas fetal human liver contains gamma globin subunits, fetal mouse liver contains beta globin. To investigate this difference, researchers sought to determine whether expression levels of BCL11A may be responsible for the different types of globin subunits between these two species. The results clearly show that BCL11A is present in much higher levels in fetal mouse liver than fetal human liver, accounting for the corresponding lack of gamma globin in the fetal mouse tissue.

These results confirm and expand scientists' understanding of the role of BCL11A in the regulation of HbF. Furthermore, this research also provides a potential therapeutic approach for HbF reactivation in patients with hemoglobin disorders.

Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, Van Handel B, Mikkola HKA, Hirschhorn JN, Cantor AB, and Orkin SH: Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science 322: 1839-1842, 2008.

Chen Z, Luo HY, Steinberg MH, and Chui DH: BCL11A represses HBG transcription in K562 cells. Blood Cells Mol Dis 42: 144-149, 2009.

Sankaran VG, Xu J, Ragoczy T, Ippolito GC, Walkley CR, Maika SD, Fujiwara Y, Ito M, Groudine M, Bender MA, Tucker PW, and Orkin SH: Developmental and species-divergent globin switching are driven by BCL11A. Nature 460: 1093-1097, 2009.

Blood Stem Cell Development with Implications for Tissue Regeneration: Scientists have recently identified key factors that interact to regulate stem cell development and tissue regeneration. Therapies to regenerate diseased or injured organs will benefit from an in-depth knowledge of how stem cells and progenitor cells develop into complex tissues and organs. Previous research has implicated several factors in the regulation of blood (hematopoietic) stem cell formation and self-renewal. These include prostaglandin E2 (PGE2) and a series of factors known as the WNT signaling pathway. However, the exact mechanism by which these factors exert their effects on hematopoietic stem cells remained unknown.

Beginning with the zebrafish model system, investigators showed that PGE2 directly increased WNT pathway activity within embryonic blood stem cells, leading to the formation of greater numbers of these stem cells. Exploring the underlying mechanisms, the researchers found that PGE2 helps stabilize an important component of the WNT pathway. In addition to demonstrating the importance of these factors to embryonic blood stem cell development, the scientists investigated a potential role for PEG2 and the WNT pathway in adult animals. By studying zebrafish whose blood stem cells had been destroyed by irradiation, the researchers discovered that these factors also work together to promote regeneration of new blood stem cells in adult fish. Further studies revealed that PGE2 and WNT work together to promote formation of embryonic and adult blood stem cells in mice—evidence that these pathways also interact in mammals. Beyond blood cells, the PGE2/WNT interaction was also shown to be required for liver regeneration in both fish and mice. These results illuminate PGE2 and the WNT pathway as powerful regulators of cellular regeneration in the body. Future research efforts will delineate the feasibility of delivering appropriate levels of PGE2 to damaged tissues to promote WNT-dependent cellular regeneration in a precise and controlled manner to preempt further injury and restore health.

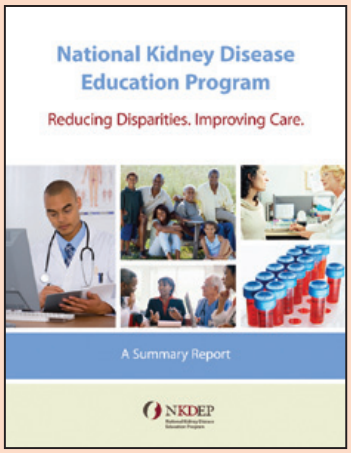
Goessling W, North TE, Loewer S, Lord AM, Lee S, Stoick-Cooper CL, Weidinger G, Puder M, Daley GQ, Moon RT, and Zon LI: Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. Cell 136: 1136-1147, 2009.

National Kidney Disease Education Program's Efforts To Improve Treatment Practices

An estimated 23 million Americans may have chronic kidney disease (CKD)[1] and, according to the NIDDK-supported U.S. Renal Data System, more than 500,000 patients are either on kidney dialysis or living with a kidney transplant.[2] Patients with CKD are at increased risk for kidney failure. It is estimated that treating the number of people with kidney failure, also called end-stage renal disease (ESRD), through dialysis or kidney transplantation costs U.S. taxpayers approximately $24 billion each year. ESRD is an enormous public health problem that disproportionately affects minority populations.

The NIDDK's National Kidney Disease Education Program (NKDEP) is helping to address these issues. This educational program seeks to raise awareness of the seriousness of kidney disease, the importance of testing people at high risk—those with diabetes, high blood pressure, cardiovascular disease, or a family history of kidney disease—and the availability of treatment to prevent or slow kidney failure. The progression from CKD to kidney failure can be prevented or delayed if it is detected and treated early enough. The NKDEP underscores that effective treatments and management strategies for kidney disease exist, yet are being underutilized.

Kidney disease is identified based on decreased kidney function or evidence of kidney damage, usually proteinuria. Kidney function is assessed through the estimated glomerular filtration rate, or eGFR. This value is derived from a mathematical formula that takes into account several factors that impact creatinine production, including age, gender, and race. Creatinine is a waste product in the blood created by the normal breakdown of muscle cells during activity. When kidneys are not working well, creatinine levels build up in the blood. Protein is the most common sign of kidney damage. Protein does not normally pass through the kidney filter into the urine. Along with eGFR, providers can measure the amount of the protein albumin in the urine (UA) to detect and plan treatment for chronic kidney disease. However, there are several issues related to standards for the measurement and reporting of UA



that have made it difficult for health care providers to use test results effectively to inform treatment decisions and monitor patients' kidney health. As a result of a March 2007 meeting bringing together members from NKDEP and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), several working groups have been formed to study the biological variability of UA and develop appropriate measurement procedures.

The NKDEP has recently released a summary report that highlights major efforts to address chronic kidney disease disparities and improve care. The report can be found at: *http://nkdep.nih.gov/resources/NKDEP_Summary_Report_508.pdf*

For NKDEP educational materials for patients, health care providers, and laboratory professionals, please visit *www.nkdep.nih.gov* or call 1-866-4 KIDNEY (1-866-454-3639).

[1] *Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, and Coresh J, for the Chronic Kidney Disease Epidemiology Collaboration: A new equation to estimate glomerular filtration rate. Ann Intern Med 150: 604-612, 2009.*

[2] *U.S. Renal Data System, USRDS 2009 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the U.S., National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2009.*

STORY OF DISCOVERY

The Promise of Induced Pluripotent Stem Cells

The NIDDK conducts and supports basic and clinical research on many of the most serious diseases affecting public health. One promising approach to prevent or treat a variety of diseases is in the recent research development of induced pluripotent stem (iPS) cells. Pluripotency is defined as the ability of stem cells to give rise to most of the various cell types in the body. In promising research, scientists are investigating the robustness of these cells as research tools, to advance understanding of disease development, and as potential sources of cells for regenerative therapies for patients.

Setting the Stage

The study of embryonic stem cells established a foundation on which to understand the properties of self-renewal and pluripotency. In 1981, the first mouse embryonic stem (ES) cell lines were isolated from embryos and grown in culture. These cells were among the first isolated cells to be shown to have the two key characteristics of embryonic stem cells: (1) They could grow and divide for long periods of time in an undifferentiated state (self-renewal)—that is, generating new stem cells, rather than specific cell types; and (2) they could also differentiate, or mature, into the many tissue- or organ-specific cell types. Human ES cell lines were established 17 years later and displayed similar properties to mouse ES cells with respect to their ability to both continually divide to form new daughter stem cells, and to develop into many different cell types.

While characterizing the transition from human ES cells into more mature cell types, NIDDK-supported researchers discovered that some genes, including *Oct4*, were turned off—suggestive of a collection of genes required for the indefinite renewal of ES cells, but no longer needed when the cell differentiates. This finding provided insight into a subset of genes that may have relevance to the renewal capability of other cell types.

Human iPS Cells—A Beginning

Building on a landmark study in mouse cells by researchers in Japan, NIDDK-supported scientists showed that differentiated skin cells derived from a human ES cell line could also be genetically "reprogrammed" to revert back to an ES cell-like state if new copies of four specific genes were introduced into the cells. The cell's own native copies of these four genes had been shut down when the cells differentiated. The scientists had engineered the new gene copies to be active and lead to production of the proteins they encoded in the differentiated cells. Once these proteins were produced in the cells, they caused the cells to be reprogrammed and to return to a stem cell-like state. Of the four proteins, one was Oct4 (encoded by the *Oct4* gene), which was known from previous research to be present in self-renewing ES cells and not in differentiating cells, as described above. The others were genes called *Klf4*, *Sox2*, and *Myc*. The reprogrammed human skin cells, or iPS cells, regained characteristics very closely resembling those of ES cells—that is, the four genes induced pluripotency. The ES-like characteristics included cell expansion essentially without limit; cell morphology (appearance) similar to ES cells; certain types of DNA modifications (called methylation) in ES cell-like patterns; and formation, in immuno-compromised mice, of a particular type of tumor observed in ES cell experiments. Remarkably, the researchers were also able to generate iPS cells from more developmentally advanced cells—including adult skin cells—by introducing two additional genes. This was quite an achievement as there had been a general belief that mature cells, such as adult skin cells, were incapable of reverting back to a less differentiated state. These findings also suggested that there may be a hierarchy

of cells within tissues—some of which can be more easily reprogrammed than others. NIDDK-supported scientists have recently reprogrammed human blood cells using the same original cocktail of *Oct4*, *Sox2*, *Klf4*, and *Myc*. This finding represents an important advance as an initial strategy to generate iPS cells to correct for diseases specific to the blood system. Importantly, a number of laboratories have been able to successfully produce iPS cells by the method of introducing a defined set of genes.

Interestingly, a high level of the reprogramming proteins need to be present for only a short period of time to initiate the genetic program of converting the mature cell to an iPS cell. During this transition, the cell begins to increase the level of additional proteins while at the same time decreasing the level of the reprogramming proteins.

Reprogramming adult human cells would not have been possible without years of prior research studying the properties of human ES cells. Two fundamental factors critical to the development of human iPS cells are based on the knowledge gained from studying human ES cells: (1) knowledge of "stemness" genes (such as *Oct4*) whose expression or repression is essential to maintain pluripotency; and (2) the delineation of conditions necessary to propagate the cells in laboratory dishes.

Potential Uses of Human iPS Cells

NIDDK-supported scientists subsequently generated iPS cell lines from patients with several different genetic diseases and disorders, providing a valuable resource for the scientific research community. These iPS cell lines were derived from cells from patients with Parkinson's disease, type 1 diabetes, Huntington's disease, Down syndrome, severe combined immunodeficiency, Gaucher's disease, forms of muscular dystrophy, and other diseases. Comparing cells differentiated from these disease-specific iPS cell lines to cells from healthy individuals may provide insights into the development and progression of these diseases. The iPS cell lines may also be useful in the laboratory to screen new candidate therapeutic agents. Additionally, with further research progress, human iPS cells might be used one day in transplantation medicine. For example, mature cells taken from a patient could potentially be reprogrammed into iPS cells and, if necessary, a defective gene corrected. Transplanting these cells back into the patient would avoid the rejection by the immune system that occurs when tissue or organs from a different person are used for transplantation. Lack of immune rejection would also negate the need for immuno-suppressive drug therapy, which carries adverse side effects. It is hoped that transplanted iPS cells might in the future be used to treat, and perhaps cure, several diseases, such as sickle cell anemia, type 1 diabetes and its complications, and liver disease.

Challenges

Modified viruses are currently used to introduce the reprogramming factors into adult cells. This process would need to be replaced with safer methods before iPS cells could be used for treatments for humans. In animal studies, the viral "vectors" are known to randomly incorporate into the genome and sometimes cause cancer. Recent NIH-supported studies have reported using alternative methods to obtain iPS cells. One such approach used a modified viral vector which can be turned on for a specific period of time to produce the necessary proteins to initiate the reprogramming and then subsequently removed almost completely from the genome. A second approach utilized a protein expression system that does not integrate into the genome and, hence, essentially eliminates the possibility of causing a genetic mutation.

The process of making iPS cells is slow and inefficient. Evidence accumulated to date suggests that the reprogramming process is influenced by the differentiation state of the target cell. During normal differentiation, as the cell matures from an early to

late stage, the genome acquires modifications in the form of DNA methylation as well as modifications of proteins called histones, which associate with and package DNA. For example, several regions of DNA that help regulate gene activity are unmethylated in early-stage cells but densely methylated in later-stage cells. Ongoing studies are investigating the use of chemicals to inhibit the methylation and histone modifications found in mature cells.

Ongoing studies also are examining the possibility of using a chemical-only approach to reprogram mature cells to iPS cells. Potentially, this means that chemicals would directly increase the levels of reprogramming proteins—thus avoiding the use of viral vectors.

Looking to the Future

Cautious optimism describes the eventual use of iPS cells for experimental models of disease, as targets in drug screening studies, and as sources for regenerating tissue. As described here, knowledge gained from studying embryonic stem cells sets the stage for the development of iPS cells. The derivation of iPS cells has led—and continues to lead—to a cascade of exciting and unexpected findings with broad implications for improving health.

Muscle Wasting in Kidney Disease and Other Conditions

Dr. William Mitch

Dr. William Mitch is the Gordon A. Cain Professor of Medicine and Director, Division of Nephrology, at Baylor College of Medicine in Houston, Texas. He received his M.D. from Harvard Medical School. Dr. Mitch is currently an NIDDK Advisory Council member and has received a MERIT award from the Institute. He is recognized as one of the world's experts on the care of patients with hypertension and chronic kidney disease, using dietary methods to protect the kidneys. In recognition of his substantial research contributions in nephrology (kidney disease) and sustained achievements in academic medicine, Dr. Mitch was awarded the prestigious John P. Peters Award in 2009 by the American Society of Nephrology. Dr. Mitch's distinguished career has largely focused on understanding the mechanisms that regulate protein metabolism, placing emphasis on how kidney disease changes protein metabolism. He has published over 250 papers. The following are highlights from the scientific presentation that Dr. Mitch gave to NIDDK's Advisory Council in February 2009.

Understanding the processes that cause the loss of muscle mass may lead to novel therapies for kidney failure, diabetes, sepsis, certain kinds of cancers, and other conditions in which muscle wasting occurs. Such knowledge also could help clinicians identify patients at greatest risk for loss of muscle mass.

The body's ability to maintain skeletal muscle tissue levels can go awry under conditions of extended bed rest, fasting, space flight, critical illness, or severe injury. Beginning with an example of the effects of bed rest, Dr. Mitch described a recent study that assessed the impact of extended bed rest on lean body mass (skeletal muscle) in healthy young and older adults. He observed that older participants lost more skeletal muscle during 10 days of bed rest than did younger participants after 28 days, suggesting that older adults requiring hospitalization for health related issues are at increased risk for significant loss of skeletal muscle.

End-Stage Kidney Disease (Kidney Failure) and Skeletal Muscle Loss

Dr. Mitch next recounted an earlier clinical finding on kidney disease that had sparked his own research interest. The 1980 study assessed the nutritional status of kidney failure patients who were undergoing dialysis, as compared to individuals with normal kidney function. When the kidneys are not functioning effectively, urea accumulates in the blood, a condition known as uremia. In some patients, this condition may progress to irreversible kidney failure, requiring dialysis—or a kidney transplant—to cleanse the body of urea and other waste products normally excreted in the urine by healthy kidneys. The results of the study were striking, showing that patients with uremia were more likely to have reduced body weight, body fat, and skeletal muscle mass in the upper, non-dominant arm. These findings confirmed what had been observed frequently for this population of patients—that wasting is common.

Dr. Mitch and his team then studied an animal model of kidney failure to investigate potential causes of muscle wasting. Because kidney failure had previously been associated with acidosis (high levels of acid in the blood and tissues), the scientists sought to determine whether acidosis promotes loss of muscle protein. When they evaluated the animals, they found that those with decreased kidney function lost muscle mass (protein) at a much greater rate than control animals. However,

when fed bicarbonate, which can help maintain proper acid-base balance in the body, the animals with impaired kidney function no longer lost muscle protein; indeed their net muscle protein loss resembled that seen in the control animals. Thus, these experiments showed that muscle protein loss in an animal model of kidney failure is influenced by acidosis that in turn can be corrected by administering bicarbonate.

What Drives the Loss of Lean Body Mass?

In further describing the link between acidosis and muscle mass, Dr. Mitch noted several studies by others. For example, when children with inherited conditions such as renal (kidney) tubular acidosis are fed bicarbonate, they grow more normally. In addition, people with kidney disease given bicarbonate do not break down more protein than usual, thereby maintaining lean body mass at normal levels. Conversely, when an acidic solution is delivered intravenously to healthy adults, they begin to break down more protein than usual.

What is the signal or trigger that causes muscle protein loss? In further studies of metabolic acidosis in uremic animals, Dr. Mitch and his team found that the pH, a measure of acid-base level, within cells was normal, not acidic. They then explored another potential trigger. Conditions such as sepsis, trauma, and inflammation, which stimulate muscle breakdown, share a common feature—insulin resistance, a state in which the body produces the hormone insulin but is unable to use it properly. Dr. Mitch and his colleagues hypothesized that defects in signaling by insulin and insulin growth factor-1 (another hormone) may cause loss of muscle protein.

Type 2 diabetes is characterized by insulin resistance. Experiments were designed to determine whether insulin resistance causes muscle breakdown in type 2 diabetes using the *db*/*db* mouse model. Three different types of muscle (soleus, extensor digitorum longus, and plantaris) from the mice with diabetes were all shown to have higher rates of protein degradation compared to their respective muscle type in normal mice, demonstrating an association between insulin resistance and increased muscle protein loss.

How Does Impaired Insulin Signaling Stimulate Protein Breakdown?

When cells break down proteins, the process most often used is called the ubiquitin-proteasome pathway. Dr. Mitch and his team speculated that this pathway is also involved in protein degradation stimulated by impaired insulin signaling. The pathway begins with the addition of several ubiquitin molecules to proteins to tag them for degradation. Tagged proteins are then recognized by a large structure, the proteasome, which chops up the proteins and releases the ubiquitin molecules to be recycled. Dr. Mitch provided evidence that the levels of ubiquitin and atrogin-1—the factor that joins ubiquitin to proteins destined for degradation—may be elevated in an experimental model of diabetes compared to control animals. Additional experiments showed that an inhibitor of proteasome function can block the increase in protein degradation caused by insulin resistance. Therefore, Dr. Mitch and his colleagues predicted that in chronic kidney disease and other diseases associated with muscle wasting, the ubiquitin-proteasome pathway is in an activated state, which contributes to increased muscle protein breakdown. He also outlined a cascade of molecular events through which impaired insulin signaling ultimately drives another cellular factor to activate the gene for atrogin-1, which in turn helps accelerate ubiquitin-proteasome activity and, hence, muscle protein degradation.

How Are Larger Muscle Protein Structures Degraded?

The myofibril is the contractile structure in skeletal muscle, and is composed mainly of the proteins actin and myosin. Reconstituted in a test tube, the

ubiquitin-proteasome system quickly degrades actin and myosin, but does not break down larger complexes of these two proteins—actomyosin or myofibrils. Metabolic acidosis places stress on cells. Dr. Mitch and his team were aware that other forms of cellular stress, for example inflammation, lead to activation of caspase-3, a type of protein that can cleave other proteins. They thus hypothesized that perhaps caspase-3 plays a role in the initial steps of muscle breakdown.

To determine whether caspase-3 is capable of the initial cleavage of muscle protein, Dr. Mitch and his colleagues added it to the actin-myosin complex (actomyosin) in a test tube. They found that caspase-3 cleaved actomyosin to generate native actin, as well as a smaller fragment of actin, which they referred to as a "14 kD actin fragment." Additional investigation revealed that the 14 kD fragment could be degraded further by the ubiquitin-proteasome system.

The 14 kD Actin Fragment as a Marker in Experimental Models and Human Disease

Dr. Mitch and his team next examined caspase-3's possible role in the initial step of increased muscle protein loss under disease conditions. Muscle tissue from animal models of acute diabetes or chronic uremia was compared with muscle from normal animals, and was found to have higher levels of 14 kD actin fragment. Additionally, more 14 kD actin fragment was detected in muscle from patients with degenerative hip disease—who are generally less mobile—and the amount of the 14 kD fragment in the muscle correlated with the measured rate of protein degradation. Thus, caspase-3 generates a characteristic 14 kD actin fragment during conditions of muscle wasting, and this fragment could be used as a biomarker of muscle protein loss.

Dr. Mitch's team then investigated whether this actin fragment reflects muscle wasting in another disease, and whether it may help in evaluating potential therapies. The impact of two types of exercise on the amount of 14 kD actin fragment in thigh muscle was evaluated in patients undergoing hemodialysis. After 18 weeks of endurance exercise, there was a decrease in the amount of actin fragment when compared to the pre-exercise period. Strength training, however, did not reduce the levels of the actin fragment after 18 weeks. These findings suggest that endurance training decreased muscle protein breakdown in patients undergoing hemodialysis, and that analysis of the actin fragment may be a useful way to assess protein degradation.

Caspase-3 Stimulates Proteasome Activity

The proteasome complex is responsible for most of the protein degradation that occurs within all cells. It includes a central core, which degrades proteins, surrounded by numerous other subunits. Dr. Mitch and colleagues suspected that these other subunits might regulate the overall activity of the proteasome.

In a series of experiments with different muscle cells, Dr. Mitch and his team found that several of the proteasome's subunits can be cleaved within the cell to increase the activity of the proteasome to degrade muscle proteins. Interestingly, these proteasome subunits are cleaved by caspase-3, the same factor that also cuts muscle proteins to prepare them for further degradation by the proteasome. To identify which subunits of the proteasome might be susceptible to cleavage by caspase-3, Dr. Mitch and his colleagues turned to a cell culture system containing either fully formed, mature muscle cells (myotubes), or immature muscle cells (myoblasts). Through experimental modulation of caspase-3 activity and analysis of the effects on various proteasome subunits, they discovered that caspase-3 cleaves different subunits in different types of muscle cells. In the immature cells, caspase-3 cuts one of the subunits and reduces proteasome activity. However, in mature muscle cells, caspase-3 cleaves different subunits, leading to an increase in proteasome activity, and thus to more muscle protein degradation.

Conclusions

Dr. Mitch's experiments have elucidated mechanisms underlying muscle wasting in chronic kidney disease and other conditions. Defective signaling by the hormones insulin and insulin growth factor-1 leads ultimately to increased activity of cellular components that degrade muscle proteins: caspase-3, the proteasome, and another factor that tags proteins for degradation. Caspase-3 both initiates muscle protein breakdown and activates proteasomes. Interventions designed to restore insulin signaling may also slow the loss of muscle protein. This research also revealed the potential beneficial use of bicarbonate to slow the loss of muscle protein in people with kidney disease. For patients undergoing hemodialysis, endurance training was shown to decrease muscle protein breakdown and thereby improve the nutritional status of the patient. Furthermore, Dr. Mitch and his colleagues have identified a marker, the 14 kD actin fragment, that has the potential to aid in the diagnosis of muscle protein wasting as well as being an indicator to assess therapeutic interventions to reverse the condition.

Dr. Mitch acknowledged the contributions of scientists who worked with him on these studies: Drs. Jie Du, Liping Zhang, Daniel Hu, S. Woo Lee, In Hee Lee, and Vik Rajan from Baylor College of Medicine; Drs. S. Russ Price and Xiaonan Wang from Emory University; Dr. A. Ferrando from the University of Arkansas School of Medicine; and Drs. Fred Goldberg and Stewart Lecker from Harvard Medical School. Dr. Mitch also indicated his appreciation of NIDDK support over the years.

A Critical Developmental Switch Controls the Onset and Progression of Cyst Formation in Polycystic Kidney Disease

Dr. Gregory G. Germino

Dr. Gregory Germino was named the Deputy Director of NIDDK in June 2009. Prior to joining NIDDK, Dr. Germino, a highly regarded physician-scientist, was a Professor in the Department of Medicine and Department of Molecular Biology and Genetics at The Johns Hopkins University School of Medicine. He received his M.D. from the University of Chicago, completed his residency in internal medicine and nephrology at Yale University, and conducted a clinical fellowship at Oxford University. Dr. Germino is a world-renowned expert in inherited kidney disease and has made seminal contributions to understanding the genetic origins of polycystic kidney disease. His research accomplishments have been recognized by several honors, including an NIH Physician-Scientist Award (1988-1993) and an NIH MERIT award (2000). At the September 2009 meeting of the NIDDK Advisory Council, Dr. Germino presented recent advances on the molecular factors contributing to the onset and progression of polycystic kidney disease; the following are highlights from his presentation.

Kidney Structure and Function

The kidneys are a pair of vital organs that keep the blood clean and chemically balanced. Blood is processed in the kidneys by millions of functional units called nephrons, where each nephron contains a filtration unit—known as a glomerulus—that is connected to a tube (or tubule) for collecting the filtered waste products. As filtered waste is collected, the tubules measure out the amounts of different chemicals present to ensure that the body maintains the right balance that is necessary for life. This important function requires tubules to have distinct cell types properly positioned with the correct orientation. If the tubule structure is compromised in some way, kidney function could be seriously impaired.

Polycystic Kidney Disease

Polycystic kidney disease (PKD) is an inherited disorder in which abnormal tubule structure leads to the formation of numerous fluid-filled cysts in the kidney. These cysts can slowly increase the mass of the kidneys and impair kidney function, leading to kidney failure. About half of people with the most common form of PKD progress to irreversible kidney failure, ultimately requiring either a kidney transplant or dialysis to survive.

In the U.S., an estimated 1 in every 1,000 people have PKD, and it is the fourth-leading cause of kidney failure. Although people with this disease have a number of options for managing pain, preventing infection, and slowing the decline of kidney function, there are no effective therapies at present to target the underlying cause of PKD progression and reverse the loss of kidney function.

Genetic Cause of PKD

Because PKD is an inherited disorder, scientists and physicians have the unique opportunity to understand the disease by studying the genetic mutations that cause it. The most common form of PKD, known as autosomal dominant PKD (ADPKD), is associated with inherited mutations in one of two genes—*PKD1* or *PKD2*. Although mutations in *PKD1* account for 85 percent of ADPKD cases, the resulting disease is clinically indistinguishable from ADPKD caused by inherited mutations in *PKD2*.

Typically, if a disease is "autosomal dominant," only one abnormal copy of a gene needs to be inherited to cause the disease. That is, of the two copies of the gene that a person inherits—one from each parent—the abnormal one "dominates." In the case of autosomal dominant PKD, however, the genetics appear to be somewhat more complicated. Although an individual with ADPKD may inherit one mutated copy of *PKD1*, for example, along with one normal copy of the gene, it is not until this normal copy acquires a mutation that cyst formation is "triggered" and disease onset begins. These acquired mutations, which occur by chance in the genes of individual cells, are called somatic mutations, to distinguish them from mutations that are inherited. (The situation is similar for *PKD2* gene mutations.) They are also referred to as "second-hit" mutations, with the first mutation being the inherited one.

By analyzing the genetic composition of kidney cysts, researchers have found that these "second-hit" mutations occur rather frequently. In addition, experiments in genetically-engineered mice with PKD mutations have shown that the severity of cystic disease is primarily determined by how quickly the normal gene copy acquires new, non-inherited mutations. Because different types of mutations impair PKD gene function to different extents, researchers have put forth a model in which the combined effects of the inherited and non-inherited mutations have to reach a certain "threshold" to trigger cyst formation.

PKD1 and _PKD2_ Gene Products and Disease

What do the *PKD1* and *PKD2* genes do, and how do they contribute to PKD onset and progress? The *PKD1* and *PKD2* genes provide the genetic information that is used to make two cellular proteins, polycystin-1 (PC-1) and polycystin-2 (PC-2), respectively. The PC-1 and PC-2 proteins are known to be important in determining the types of cells that are formed in kidney tubules and are critical for tubule development and structure.

PC-1 and PC-2 are large proteins that sit on the surface of tubule cells and form a "receptor-channel complex." A large portion of PC-1 extends outward from the cell surface and acts as an antenna (or receptor) to sense (receive) chemical, cellular, or mechanical stimuli. Detection of these stimuli by PC-1 is then coupled—or transmitted—to PC-2, which acts as a channel to let calcium, an important cellular messenger, flow into the cell. It is believed that this sense-and-respond signaling is important in mediating interactions between cells, as well as interactions between a cell and the matrix of molecules lining the extracellular surface. In addition, the PC-1/PC-2 receptor-channel complex is also found on hair-like protrusions, known as cilia, which extend from the cell surface into the interior of tubules, where liquid flows. It is on the surface of cilia where PC-1/PC-2 sends signals back to the cell in response to either the mechanical forces it feels while fluid flows through the tubule or to some other undefined trigger. Disruption of normal cilia signaling through mutations in *PKD1* or *PKD2* that alter PC-1/PC-2 function may be an underlying contributor to PKD.

Life Stage Determines Response to Loss of _PKD1_

Although the PC-1/PC-2 complex is important for establishing tubule structure during kidney development, researchers are not certain what role PC-1/PC-2 plays in maintaining tubule structure in the adult (developed) kidney. As such, an important clinical question relates to when *PKD1* and *PKD2* are required for kidney function: Are mutations that occur in these genes during adulthood sufficient for triggering cyst formation, or do these inactivating mutations (and cyst formation) need to occur at an earlier life stage when the kidneys are still developing? To address this important question regarding the onset of PKD, Dr. Germino and his colleagues have generated genetically-engineered mouse models of PKD in which they can turn off the *PKD1* gene in a controlled manner at various time points. By looking at how "knocking out" *PKD1* during different stages of development affects cyst

formation, they can evaluate the role of *PKD1* in the developing and adult kidney.

Initially, Dr. Germino's team looked at the effect of *PKD1* inactivation in newborn, adolescent, and adult mice. When the researchers inactivated *PKD1* in mice 2 days after they were born, the mice developed severely cystic kidneys quickly, within a couple of weeks. In contrast, if the researchers waited until the mice were adolescents or adults before inactivating *PKD1*, it took at least 5 months for cysts to develop. Since kidneys are fully developed in adolescents and adults and *PKD1* inactivation at that point still results in cyst formation, these results indicate that *PKD1* is important for maintaining normal tubule structure during adulthood. Interestingly, however, the progression of cyst formation was unexpectedly different following *PKD1* inactivation during different life stages.

To better define the developmental period that controls the differential response to loss of *PKD1* function, the researchers inactivated *PKD1* in mice at various intervals between 2 and 21 days after birth. If *PKD1* was inactivated when the mice were 12 days old or younger, their kidneys developed cysts very rapidly—within a couple of weeks, as was seen with the mice whose PKD genes were inactivated just 2 days after birth. Remarkably, when *PKD1* was inactivated 14 days or more after birth, the kidneys of the mice all appeared normal for 3 months but then developed late-onset cystic disease by 6 months. Examining the molecular and genetic differences that may contribute to this difference in timing of cyst formation, the researchers found that the rate of cellular growth and division in the kidneys drops abruptly at 16 days after birth. Paralleling this change in cellular growth, the cells underwent dramatic changes in genetic activity between 12 and 14 days after birth, with over 800 different genes becoming more or less active during this period. These results identify an important developmental switch that is being triggered during this critical time frame.

From these experiments in mice, Dr. Germino proposed that, in addition to the rate of acquiring mutations that inactivate the once-normal copies of the *PKD1* gene, the timing of *PKD1* inactivation is another critical factor for determining the rate of cyst growth and disease onset.

Future Directions and Clinical Implications

At this point, it is not exactly clear what accounts for the life-stage specific response to *PKD1* inactivation. Although the rate of cellular growth drops abruptly at day 16, the relationship between cyst formation and cell growth is rather complicated; the decline in cellular growth may reflect completion of the final stages of tubule maturation. In addition, the rate of disease progression does not appear to be due to disruption of PC-1/PC-2 as the cilia's mechanical sensor. Although flow sensing may be important during kidney development, the long interval between loss of PC-1 and cyst formation in adults suggests that a dynamic process like fluid flow would not be the cause of disease onset. Further experiments will be needed to fully understand the role of PC-1 at different life stages—such as its role in orienting cells after cell division—and how it contributes to the late-onset of cyst formation following inactivation in adults.

Understanding why *PKD1* inactivation in adult mice leads to late-onset cyst formation has important clinical implications for human disease. Dr. Germino's studies suggest that the underlying molecular causes may differ for early-onset and late-onset PKD, with the late-onset mouse model reflecting the gradual onset and slow progression of ADPKD in humans. To date, however, most testing of potential therapies has been done in mouse models with relatively rapid rates of disease onset and progression. Thus, it is not clear whether such studies would apply to human disease. As there are currently no therapeutic options available, the late-onset disease models developed by Dr. Germino may provide a better tool for testing new therapies in ADPKD.

PATIENT PROFILE

Janet Colardo

Benefits of Weight Loss in Reducing Urinary Incontinence in Overweight or Obese Women

Janet Colardo

An estimated 13 million Americans, most of them women, suffer from urinary incontinence (UI). Encouraging research sponsored by the NIDDK and the NIH Office of Research on Women's Health has recently shown, however, that women who are overweight or obese can significantly reduce their episodes of UI by losing weight.

Fifty-three-year-old Janet Colardo says she has always been overweight, and although she might not have thought of it as UI, she cannot remember a time when she didn't leak urine involuntarily, especially when someone or something made her laugh. "I've always been one of those pear-shaped people," says Janet. "In high school, when my classmates squeezed into designer jeans, I just couldn't do it." Janet also says that she was always asking her teachers for permission to go to the bathroom.

For decades Janet considered her urinary condition normal. "I figured it was just who I was." Then in 2006, she noticed an ad in her local newspaper for a study on weight loss and UI. The timing was perfect. "One of my sons was getting married, and I wanted to lose weight for the wedding," Janet says. "That was a big motivator for me. I figured if I could lose weight and learn how to control my bladder at the same time, all the better." Janet was interviewed and accepted into the study.

Reducing urinary incontinence can now be added to the extensive list of health benefits of weight loss, according to a clinical trial funded by the NIDDK and the NIH Office of Research on Women's Health.

PRIDE

The study Janet volunteered to participate in was called the Program to Reduce Incontinence by Diet and Exercise, or PRIDE.

Conducted in Birmingham, Alabama, and Providence, Rhode Island, PRIDE researchers recruited a total of 338 overweight and obese women who experienced UI episodes at least 10 times per week. The women were randomly assigned to either an intensive 6-month weight-loss program of diet, exercise and behavior modification or to a group that received information about diet and exercise, but no training to help them change habits.

PATIENT PROFILE

PRIDE researchers reported that women in the intensive weight-loss group lost an average of 8 percent of their body weight (about 17 pounds) and reduced UI episodes by nearly one-half (47 percent). In contrast, women in the information-only group lost an average of 1.6 percent of body weight (about 3 pounds) and had 28 percent fewer episodes. Further analysis of the data showed that 41 percent of the women in the intensive weight-loss group achieved a clinically relevant reduction of at least 70 percent of total incontinence episodes per week, whereas only 22 percent of women in the information-only group achieved the same level of reduction.

PRIDE showed conclusively that weight loss has a significant, positive impact on reducing UI in women. This finding may help motivate weight loss, which has additional health benefits for those who are overweight or obese. One such benefit is prevention of type 2 diabetes. In fact, the intensive weight-loss intervention in PRIDE was modeled after the lifestyle interventions in the Diabetes Prevention Program (DPP) and the ongoing Look AHEAD (Action for Health in Diabetes), two NIDDK-sponsored clinical trials of people who are at risk for or who have type 2 diabetes, respectively.

Participating in the Study

Janet entered the program weighing 193 pounds and completed it 6 months later weighing in the mid 170s. "I went right down in my weight," says Janet, who is 5 feet, 7 inches tall. She also reports that her incidence of UI dropped significantly.

"The PRIDE staff was extremely supportive. They provided us with all the information and tools we needed, and it was up to us to put them to good use."

For example, each participant in Janet's group was informed about proper eating habits and was issued a journal to document daily caloric intake. They also were shown how to properly perform Kegel exercises to strengthen their pelvic floor muscles, which when weakened as a result of excessive weight or other conditions (*e.g.*, pregnancy, childbirth, and aging) can lead to UI.

"The PRIDE staff was extremely supportive. They provided us with all the information and tools we needed, and it was up to us to put them to good use."

Janet admits that she never really exercised before entering the study. However, she says she now has a greater respect for the many benefits of exercise and living a healthy lifestyle, which she says the PRIDE staff instilled in her. To encourage more daily activity, Janet and her fellow participants were given pedometers to measure the distance they walked each day.

Other NIDDK-sponsored research has shown the link between excessive body weight and diabetes. Since the program ended, Janet says she has been dealing with endocrine problems that are making it difficult for her to lose weight, and she has put on some of the weight she lost. Fortunately, however, she hasn't been diagnosed with diabetes.

"If you tend to be on the heavy set side, you're always struggling to lose weight," says Janet. "The difference for me now is that because of the PRIDE program I know how to lose weight, and I know I can be successful at it."

About Urinary Incontinence

UI is caused when the bladder muscles squeeze too often—or when you don't want them to. It can also occur if muscles around the bladder opening are not strong enough to hold back urine. Women usually experience "stress" or "urge" UI, and sometimes both. Stress UI is the leakage of small amounts of urine during physical activity, such as coughing, sneezing, and exercising. Urge UI is the leakage of large amounts

PATIENT PROFILE

of urine at unexpected times, including during sleep. Janet says she has experienced both types, but fortunately never has had an incident while sleeping.

Understandably, UI is associated with diminished quality of life. But, the good news is that the symptoms of UI can often be treated, and thanks to the PRIDE study it is now confirmed that losing weight can now be included as one of the treatment approaches that should be considered for women who are overweight or obese.

As for Janet, she says she would "absolutely" recommend studies like PRIDE to others. She says that the PRIDE staff made her feel accountable to herself, as well as to them. "And I'll have the knowledge they gave me forever," she says.

For additional information on UI—

For Women:
http://kidney.niddk.nih.gov/kudiseases/pubs/uiwomen/index.htm

For Men:
http://kidney.niddk.nih.gov/kudiseases/pubs/uimen/

For Children:
http://kidney.niddk.nih.gov/kudiseases/pubs/uichildren/

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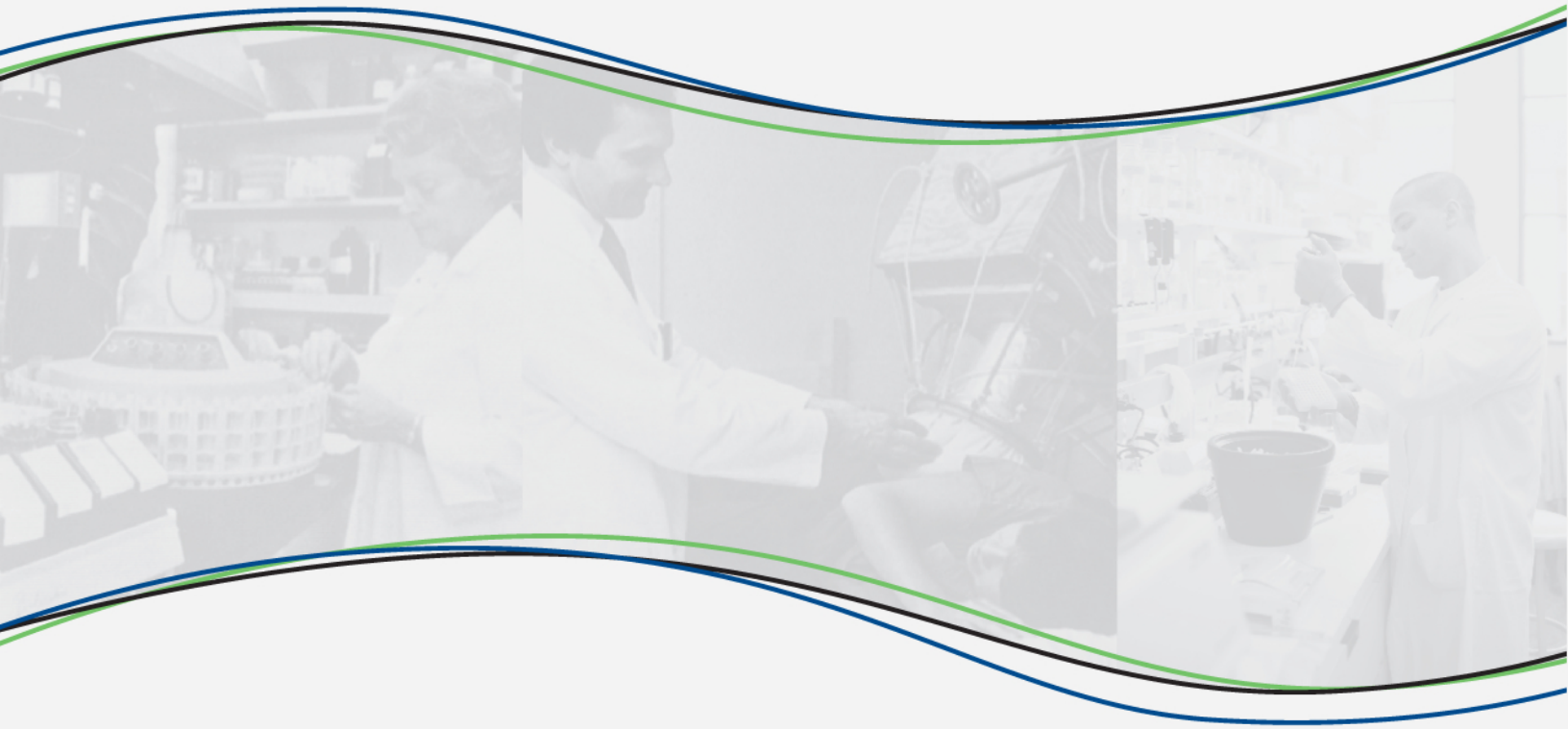

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